Protease inhibitor plasma concentrations in HIV antiretroviral therapy

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INTRODUCTION

Since the introduction of the first HIV protease inhibitor (PI), saquinavir, in 1995 (FDA approval), considerable progress has been made in the treatment of HIV-infected patients [1, 2]. In 1997, randomized controlled trials documented the superiority of HAART, highly active antiretroviral therapy, including a PI and two nucleoside reverse transcriptase inhibitors, NRTIs, compared to dual NRTI therapy [3, 4]. The CD4 cell count and plasma HIV RNA had been established as prognostic markers in patients who did not receive antiretroviral therapy but also in patients initiating antiretroviral therapy [5-9]. Treatment with HAART resulted in suppression of viral replication (plasma HIV RNA below the limit of detection) and restoration or preservation of the immune system (CD4 cell count) and these changes were associated with declining morbidity and mortality rates among HIV-infected patients [6-8].

In the AIDS Clinical Trials Group (ACTG) study 320 from 1997 including patients with a CD4 cell count of 200 cells/µl or less, the proportion of patients with plasma HIV RNA <500 copies/ml at week 24 were 60% in the group treated with the PI indinavir and two NRTIs compared with 9% in the group treated with dual NRTI therapy [3]. Corresponding to these findings the proportion of patients whose disease progressed to AIDS or death was lower in the indinavir group (6%) compared with the dual NRTI group (11%) after a median follow-up of 38 weeks.

In Denmark (1999), retrospective results from two centres evaluating HAART showed response rates (plasma HIV RNA <200 copies/ml) as high as 65% at week 48 in a heterogeneous population of dual NRTI-experienced and treatment-naïve patients [10, 11]. However, differences between the two centres in the number of virological failures were noteworthy, 0% (0/61) versus 21% (34/163), despite similar patient baseline characteristics and treatment failure criteria. The reason was most likely the choice of first-line PI which was quite different between the two centres. One used mainly indinavir, 79% (48/619), while the other used mainly saquinavir, 85% (138/163).

Although saquinavir exhibits high *in vitro* potency against HIV, it was already known at that time (1999) that the bioavailability of the saquinavir hard-gel capsule (HGC) was low (4%) and plasma concentrations highly variable [12-15]. This was believed to be a cause of the transient response or lack of response to saquinavir therapy observed in many patients [16, 17]. As a consequence, studies with higher doses of saquinavir were performed, e.g. by Schapiro et al in 1996, showing improved efficacy, and that efficacy correlated with saquinavir plasma concentrations [18]. A new formulation of saquinavir, the soft-gel capsule (SGC), was developed to improve bioavailability, and a dose-ranging study established a concentration-efficacy association which was used to optimise the dose of the saquinavir SGC [19, 20]. In 1997, another approach was investigated by Merry et al [14]. A pharmacokinetic drug-drug interaction with another PI, ritonavir, was exploited to increase concentrations of saquinavir.

A concentration-efficacy association was also observed in 1995 by Danner et al in a phase I/II study of different doses of ritonavir [21]. An *in vitro* 90% effective concentration of 2100 ng/ml against HIV-1 type IIIB (wild-type virus) in MT4 cells, after adjustment for protein binding, had been estimated. The study demonstrated that only patients with minimum concentrations above this concentration had long-term effects on plasma HIV RNA [21]. Conversely, the frequency of adverse events (nausea and elevated hepatic enzymes) increased with higher doses and corresponding higher ritonavir concentrations. This was also demonstrated in 1999 by Gatti et al [22]. In this study, patients with ritonavir-associated gastrointestinal and neurological adverse events had at least a 3-fold higher concentration than 2100 ng/ml (IC_{90}). The study conclusion included a proposal to use drug monitoring to guide drug downward titration in these patients.

In studies with HIV-infected patients receiving indinavir at a dose of 800 mg three-times-a-day, treatment was associated with urological symptoms in approximately 8% of patients [23]. It was demonstrated that the urological symptoms were associated with higher indinavir plasma concentrations (×2.64 above the mean) and that concentration-controlled dose reductions could be used to eliminate symptoms in some patients [23, 24]. However, too low indinavir plasma concentrations were also recognized as a problem. In a study by Burger et al, lower indinavir concentrations were related to virological treatment failure. The study showed that the indinavir concentration should be at least 100 ng/ml to optimise virological response [25].

Also, conference proceedings from 1997 to 2000 reported of associations between plasma concentrations and efficacy for the PI nelfinavir, which had also been approved for clinical use, and two other PIs, amprenavir and lopinavir, which were in the accelerated drug approval process [26-28]. An active nelfinavir metabolite, M8, which possessed *in vitro* antiretroviral activity comparable to that of nelfinavir had been identified but the role of M8 *in vivo* was unknown [29].

Other studies from 1997 to 1999, reported of significant changes in PI pharmacokinetics because of drug-drug interactions between PIs and co-administered drugs and the possible implications for efficacy and toxicity was a topic for discussion [30, 31]. A new class of drugs against the reverse transcriptase, the non-nucleoside transcriptase inhibitors, NNRTIs (nevirapine, delavirdine and efavirenz), had been introduced as a part of HAART and clinical significant drug-drug interactions between NNRTIs and several of the PIs had been reported [32]. As a consequence, several HIV researchers/clinicians had begun to discuss the use of PI concentration measurements to optimise HIV antiretroviral therapy (therapeutic drug monitoring) [15, 22].

OBJECTIVES

The objectives of this study, initiated in 2000, were:

- to establish a method for the simultaneous measurement of the available PIs (saquinavir, ritonavir, indinavir, nelfinavir, amprenavir and lopinavir) and the nelfinavir active metabolite M8 [II] (atazanavir, 2004)
- to explore the pharmacokinetics of the PIs in clinically relevant situations [I, III-VIII]

and in this context:

– to consider the applicability of therapeutic drug monitoring (TDM) in PI therapy [VIII].

PROTEASE INHIBITORS

PROTEASE INHIBITOR PHARMACODYNAMICS

The pharmacodynamics of HIV protease inhibitors can be divided in the intended pharmacological effects on HIV and the unintended toxicological effects on the human body.

HIV

The HIV genome is composed of three genes, *gag*, *pol* and *env*. Translation of the *gag* and *pol* gene results in two large precursor polyproteins, p55 (*gag*) and p160 (*gag-pol*). The HIV protease is responsible for the cleavage of these polyproteins (proteolytic processing) to structural proteins (p55: matrix, capsid, nucleocapsid) and replicative enzymes (p160: protease, reverse transcriptase, integrase). It was shown around 1990 in *in vitro* studies that the substitution or removal of amino acids in the HIV protease, by mutations or deletions in the protease gene, eliminated the function of the HIV protease and resulted in the formation of non-infectious HIV [33, 34]. Further *in vitro* studies demonstrated that synthetic compounds could inhibit the HIV protease with similar results [35, 36].

The available HIV PIs act by binding to the catalytic site of the HIV protease and inhibit proteolytic processing. Consequently, the PIs prevent the production of infectious HIV and the infection of new cells but have no effect on cells with integrated proviral HIV DNA. To be active against the HIV protease, the HIV PIs have to be located intracellularly, although the pharmacological effect is probably partly exerted in HIV which has already been released from the cell [35]. As with other drugs, it is only the free fraction of drug (unbound) which is available for influx into the cell and subsequently can exert the pharmacological effect (discussed below).

Human body

Many unintended toxicological effects (side effects) have been reported following the introduction of the PIs. Nausea/vomiting, diarrhoea and lipodystrophy are believed to be common for this class of drugs while other side effects are more or less specific to individual PIs e.g. nephrolithiasis (indinavir and possibly atazanavir) and circumoral paresthesia (ritonavir and amprenavir) [III, VIII, 21, 23, 37-39]. The exact mechanisms behind most of the PI-related side effects are not known in detail apart from indinavir-associated nephrolithiasis, which is most likely caused by precipitation of indinavir in the renal tubules [23].

PROTEASE INHIBITOR CONCENTRATION MEASUREMENT

To study PI pharmacokinetics, the availability of methods that can measure PI concentrations with accuracy and precision is required. To select the right method for pharmacokinetic purposes preanalytical, analytical and postanalytical aspects have to be considered. Postanalytical aspects will be discussed below in the section about PIs and TDM.

Preanalytical aspects

Preanalytical aspects of PI concentration measurement include time of blood sampling and processing and storage of the sample [40]. The precise time of PI administration in relation to the time of blood sampling must be available if results should be compared and interpreted. The time of day of the blood sample is also important as diurnal variation of PI concentrations have been described with all PIs administered twice-a-day with the morning $C_{through}$ being considerable higher than the evening $C_{through}$ (ratio range: 1.3-2.9) (Table 1) [I, 41]. Plasma is the preferred matrix for PI measurement. The stability of PIs in whole blood samples has not been fully elucidated and the time from sampling to centrifugation might be critical. In general it is recommended that blood is processed within 2 hours of collection [42]. It is possible that drug influx or efflux by transport proteins in peripheral blood mononuclear cells (PBMC) could influence the result of plasma concentration measurements. However, in our own study we did not find any significant difference between plasma concentrations of saquinavir, ritonavir, indinavir, nelfinavir, amprenavir, lopinavir or M8 from whole blood samples kept at room temperature (20°C) or in a refrigerator (4°C) for 6 hours before centrifugation compared with the concentrations from samples which were immediately centrifuged [II]. The mean and median ratio was 1.03 at 4°C and 1.03 at 20°C (n=85) [II]. It has also been shown in a study with [^{14}C]radiolabelled nelfinavir that very little radioactivity was found in erythrocytes suggesting that there is probably no influx or efflux from these cells [43]. Several studies have shown that PIs are stable in plasma for at least 6 months at –20°C, 7 days at 4°C, 24 hours at 20°C and 1 hour at 60°C [II, 44, 45]. It has also been demonstrated that PI plasma concentrations do not change despite several (3-4) freeze-thaw cycles [II, 44]. In conclusion, PI concentrations are very stable under different circumstances in whole blood and plasma.

Analytical aspects

Several high-performance liquid chromatography (HPLC) methods for PI concentration measurement have been published. Before 2000, most of them included only a single or two protease inhibitors [46-48]. As the number of PIs increased and the combination of two

Table 1. Diurnal variation of protease inhibitor concentrations with twice-a-day administration.

Protease inhibitor	Co-administered PI or NNRTI	N	Morning $C_{through}$/ evening $C_{through}$ ratio	References
Saquinavir	Ritonavir	4	2.0[b]	Justesen et al [I]
	Ritonavir and lopinavir	25	1.5	Ribera et al [144]
Ritonavir	None	46	1.5	Hsu et al [96]
	Saquinavir and lopinavir	25	1.3	Ribera et al [144]
Indinavir	Ritonavir	7	1.4[b]	Justesen et al [I]
	Ritonavir and efavirenz	5	1.4-3.3[c]	Lee et al [218]
	Ritonavir	19	2.9	Boyd et al [140]
Nelfinavir	None[a]	355	2.5	Baede-van Dijk et al [43]
	None	12	2.4	Ford et al [130]
Amprenavir	Delavirdine	18	1.6[b]	Justesen et al [I]
Lopinavir	Ritonavir	11	1.4	Crommentuyn et al [41]
	Saquinavir and ritonavir	25	1.3	Ribera et al [144]

PI: protease inhibitor. NNRTI: non-nucleoside reverse transcriptase inhibitor.
a) Some patients received once-a-day dosing of a drug which could interact with nelfinavir (efavirenz, rifabutin, omeprazole).
b) Data are not reported separately for each PI in the paper [I].
c) Three different doses of indinavir were examined.

or more PIs became frequent in antiretroviral therapy, supported by data from clinical trials, methods for the simultaneous measurement of all available PIs were developed [II, 44, 45, 49-51]. The simultaneous measurement of many PIs is to prefer considering throughput and cost effectiveness giving acceptable analytical quality.

Pretreatment with either protein precipitation, liquid-liquid or solid-phase extraction is used followed by separation with isocratic or gradient elution on a C_8 or C_{18} column as the stationary phase (e.g. ion-pair or reverse phase) [II, 46, 48, 51]. Detection is most often UV-detection.

During the evaluation of an analytical method, analytical specificity, selectivity, precision and accuracy have to be considered [II]. The analytical specificity is particularly important when measuring PIs in HIV-infected patients as these patients receive other drugs than PIs. Therefore, most of the methods used for PI measurements have been evaluated in a clinical setting to exclude interference from co-administered drugs and possible metabolites. In our own study, we tested all available antiretroviral drugs (zidovudine, lamivudine, didanosine, stavudine, abacavir, zalcitabine, delavirdine, nevirapine, efavirenz) and other frequently co-administered drugs (e.g. antibacterial, antifungal and other antiviral drugs) for interference in samples obtained from patients. Drug-free plasma samples were also tested to exclude interference from endogenous substances [II]. No interference was observed [II].

To maintain good quality, it is recommended that control samples are included in every analytical run (intra-laboratory quality control), and this is the practice in most published methods [II, 44, 45, 51-53]. The use of internal standards, which are added to every sample, to enhance performance is also applied in some of the published methods, but with more than seven compounds in the same analysis it might be difficult to find a suitable internal standard which will not co-elute with one of the compounds of interest [II, 44, 51]. To ensure acceptable analytical accuracy the regular participation in external quality control programmes is also encouraged [53]. National (Asqualab program, France) and international inter-laboratory quality control programmes are running in several, primarily European, countries. The *International Interlaboratory Quality Control Program for Therapeutic Drug Monitoring in HIV infection* which is directed from the Departments of Clinical Pharmacy, University Medical Centre, Nijmegen, The Netherlands, has published results from their program [54, 55]. In the third round from 2001 only three laboratories, including our Danish laboratory, out of 30 laboratories, performed all their measurements within the acceptance range (80%-120% accuracy). Another 12 laboratories reported at least 90% of their measurements within the acceptance range [55]. The study demonstrated the usefulness of participation in a quality control programme to enable laboratories to take action with subsequent improvement in performance [55]. The acceptance range (accuracy) and the inter-assay or between-day variation (precision) of a method should be determined and evaluated in relation to the intra-individual variation of PI concentrations in HIV-infected patients (30-45%) if the method is used for TDM purposes. The inter-assay variation must be considerably lower than the intra-individual variation to correctly evaluate PI concentration measurements.

The necessary limit of quantitation of the method will depend on the context in which the method is used. For TDM purposes, a limit of quantitation of 100 ng/ml will be sufficient in most cases [II]. For pharmacokinetic studies beyond the dosing interval or for measuring PI concentrations in other matrices than plasma, e.g. cerebrospinal fluid or intracellular concentrations, a lower limit of quantitation is required. HPLC methods can be optimised to obtain limits of quantitation as low as 10-25 ng/ml with coefficients of variation (CV) of less than 10-20% [II]. An approach to further enhance sensitivity is to use detection by mass spectrometry [47].

PROTEASE INHIBITOR PHARMACOKINETICS

Absorption

Data on the bioavailability of the PIs are incomplete but can vary from 4%, saquinavir HGC, to 65% for indinavir (Appendix 1). All the PIs are subjected to varying degrees of first-pass metabolism, especially saquinavir, due to varying affinity to the drug-metabolising enzyme system cytochrome P450 and the drug-transporting protein P-glycoprotein in both the small intestine and in the liver [56-59]. First pass metabolism can be reduced if PIs are co-administered with a drug-metabolising enzyme inhibitor (discussed below). The absorption of nelfianvir is highly dependent on the concomitant intake of food. Nelfinavir AUC values can be 50% higher with food than during fasting conditions [60]. It is recommended that saquinavir, lopinavir and atazanavir are administered with food but there are no food requirements with ritonavir or amprenavir. In contrast, absorption of indinavir, administered as the only PI, is reduced when taken with food. The t_{max} for PIs differs as well. Indinavir and amprenavir are absorbed very rapidly, with a t_{max} of 1-2 hours. The amprenavir t_{max} in fasting healthy volunteers can be even shorter (45 minutes) [III]. A second peak or plateau 6-12 hours after administration of amprenavir has been reported and is believed to be caused by enterohepatic circulation of amprenavir [III, 39]. Saquinavir, ritonavir, nelfinavir, lopinavir and atazanavir all have a t_{max} of 2 hours or more (Appendix 1).

Distribution

PIs distribute into most body compartments, including lymphoid tissue. The central nervous system and testis seem to be pharmacological sanctuary sites with only indinavir penetrating the blood-brain and blood-testis barrier in therapeutic concentrations (discussed below) [61-65].

Protein binding

All the PIs except indinavir (61%) are highly protein-bound, 86% or more (Appendix 1). Plasma protein binding is primarily to α1-acid glycoprotein (orosomucoid), although atazanavir also binds to albumin. It is only the free fraction of PIs (unbound), which is available for influx into the cell and subsequently can exert the pharmacological effect. PI concentration-dependent changes in protein binding and protein concentration-dependent changes in PI concentrations have been investigated partly *in vivo* and *in vitro* to assess the effects and possible implications for PI therapy.

In two *in vivo* studies by Boffito et al, total and unbound lopinavir, saquinavir and indinavir concentrations were examined over a dosing interval and concentration-dependent binding of lopinavir was demonstrated [66, 67]. The unbound fraction of lopinavir was higher 2 hours post dose than at baseline (1.05% versus 0.84%) corresponding with higher total concentrations of lopinavir whereas this was not the case with either saquinavir or indinavir. However, in another study by Anderson et al, concentration-dependent changes in protein binding of indinavir were demonstrated, with lower fractions of unbound indinavir (34% versus 43%) at the end of the dosing interval, 8 hours post dose (low total concentrations), compared with 1 hour post dose (high total concentrations) [68]. Still, all three studies demonstrated that the AUC and the concentrations at different time points of unbound and total PI overall were very well correlated which means that the total drug concentration can be used as a marker for unbound lopinavir, saquinavir or indinavir regardless of the concentration level [66-68]. The same has been observed with atazanavir [37].

Numerous *in vitro* studies have shown that the efficacy of PIs decreases with increasing concentrations of α1-acid glycoprotein [69-71]. It has been shown that the effective concentration that produced 50% of the maximal antiretroviral effect (EC_{50}) against wild type HIV in 0% human serum compared with 50% human serum increased 35-fold (nelfinavir), 25-fold (saquinavir), 19-fold (ritonavir), 6-fold (amprenavir) 5.3-fold (lopinavir) and 2-fold (indinavir)

corresponding partly to what would be expected from *in vivo* protein binding data (Appendix 1) [69].

Likewise, it was shown *in vitro* by Jones et al that the intracellular concentrations of saquinavir, ritonavir and indinavir were reduced with increasing α1-acid glycoprotein concentrations indicating a lower free fraction [71]. However, in the discussion of the study, it was also pointed out that as total PI concentrations will increase with increasing α1-acid glycoprotein concentrations and because PIs are high clearance drugs, the free PI concentration and the intracellular concentration will probably be unaffected *in vivo* [71]. α1-acid glycoprotein concentrations have been shown to vary and mimic a normal distribution, within a population of HIV-infected patients (n=81), range: 15-170 mg/dl, median 79 mg/dl [72]. This means that some patients with very high or very low α1-acid glycoprotein concentrations will show corresponding high or low total PI concentrations whereas the unbound concentration will not be affected [73]. The relationship between clearance and α1-acid glycoprotein concentrations was investigated by Sadler et al in an *in vivo* study with amprenavir including both HIV-infected patients (n=18) and healthy volunteers (n=68) [74]. In this study an inverse relationship was found between α1-acid glycoprotein concentrations and amprenavir clearance. As described, the unbound fraction (f_u) of PIs decreases with increasing α1-acid glycoprotein concentrations. In case of high clearance drugs with predominantly hepatic metabolism (such as the PIs), the relationship can be expressed with the equation [$CL = f_u \times CL_{int}$] where total body clearance (CL) is proportional with the unbound fraction (f_u) as the CL_{int} (intrinsic clearance of drug from plasma by the liver devoid of influence of blood flow or protein binding) is considered a constant [73]. The same inverse relationship has been demonstrated between α1-acid glycoprotein concentrations and saquinavir clearance in mice and indinavir and lopinavir clearance in humans [75, 76].

The clinical implications of PI protein binding are not clear. It is possible to make the wrong assumption about PI exposure based on total drug concentrations in patients with either very high or very low α1-acid glycoprotein concentrations, but efficacy is not expected to be affected by protein binding as such because the free PI concentration will largely remain unchanged.

However, a problem with potential clinical implications has been the different approaches used by the PI manufacturers to determine protein binding and efficacy terminology ($EC_{\#\#}$ or $IC_{\#\#}$) which has made it very difficult to compare the potency of the different PIs [77]. The IC_{50} is defined as the inhibitory concentration that exhibits 50% of the maximal antiretroviral effect in an *in vitro* system. However, the level of protein binding in the *in vitro* system could vary from low levels of protein binding (10-20% foetal bovine serum) to near *in vivo* levels (10% foetal bovine serum with 50% human serum) [78]. Some has used the term effective concentration (EC) which is determined in 50% human serum with higher levels of protein binding [69]. However, ECs are also used to describe effective concentrations from *in vivo* studies (discussed below) [20, 39].

Metabolism and excretion

Cytochrome P450

The metabolism of the PIs is primarily oxidative by the cytochrome P450 (CYP) system in the small intestine (minor) and in the liver (major), (Figure 1) [56, 61, 79, 80]. It is predominantly the isozyme CYP3A4 which is responsible for the metabolism of saquinavir,

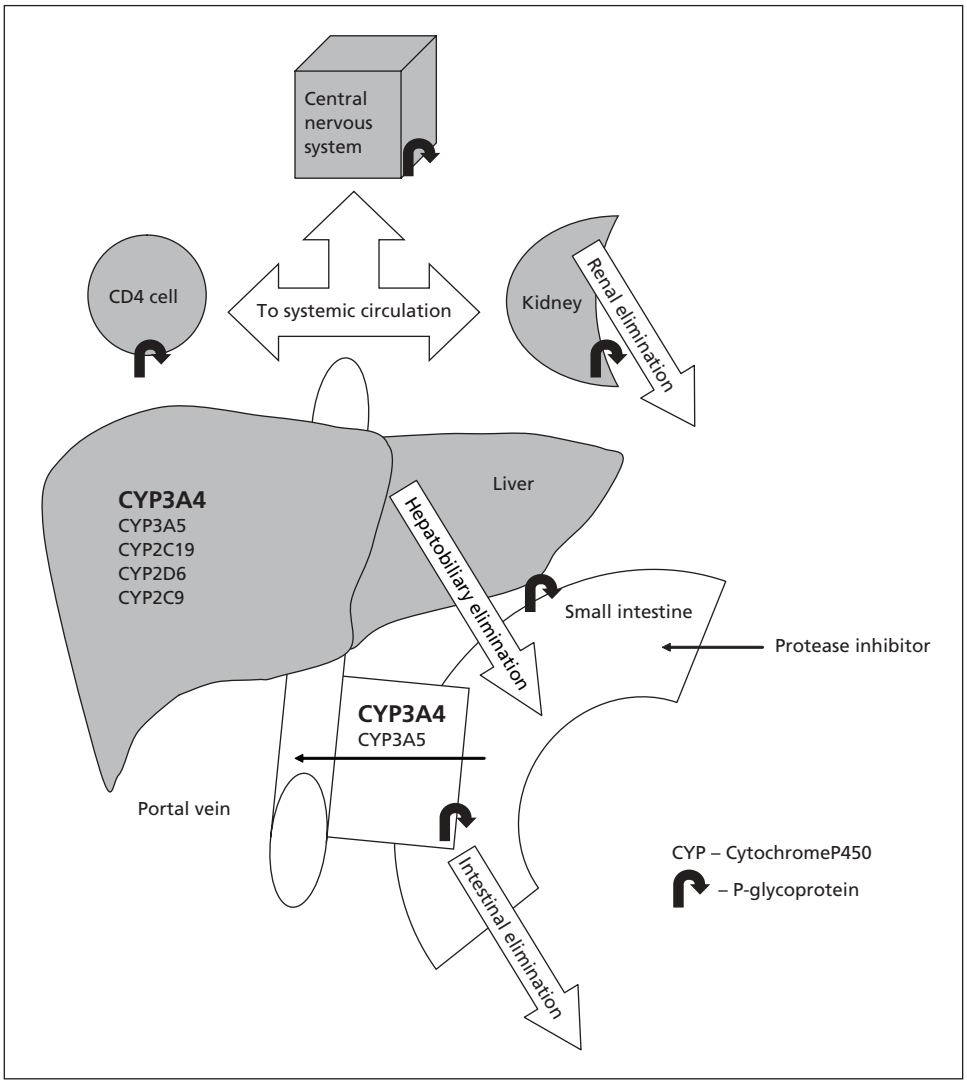

Figure 1. Protease inhibitor metabolism and excretion by the cytochrome P450 system and P-glycoprotein system.

Table 2. Long-term intra-individual variation of protease inhibitor concentrations.

Protease inhibitor	Co-administered PI or NNRTI	N	Median $C_{through}$ (ng/ml) and weeks between	P-value	References
Saquinavir	Ritonavir	6	400 and 259 (40)	0.06	Gisolf et al [1 13]
	Ritonavir	89	1035 and 912 (44)	0.25	Justesen et al [VIII]
Ritonavir	Saquinavir	89	416 and 434 (44)	0.85	Justesen et al [VIII]
	Indinavir	34	680 and 548 (44)	0.40	Justesen et al [VIII]
	Lopinavir	44	218 and 232 (44)	0.58	Justesen et al [VIII]
Indinavir	Ritonaivr	34	1389 and 1230 (44)	0.21	Justesen et al [VIII]
Nelfinavir	Ritonavir	9	746 and 773 (20)	0.43[a]	Justesen et al [VII]
Amprenavir	Delavirdine	5	595 and 595 (26)	NR	Engelhorn et al [207]
Lopinavir	Ritonavir	44	5531 and 5821 (44)	0.68	Justesen et al [VIII]

PI: protease inhibitor. NNRTI: non-nucleoside reverse transcriptase inhibitor. NR: not reported.
a) The P-value is not reported in the paper (VII).

ritonavir, indinavir, amprenavir, lopinavir and atazanavir, although other isozymes are also involved, e.g. CYP2D6 (ritonavir) [37]. CYP3A4 is present in the small intestine and contributes to the poor bioavailability of the PIs, especially saquinavir [56]. Hepatic CYP3A4 displays significant inter-individual variability, which has been shown to be accentuated in HIV-infected patients. In a study with 47 healthy volunteers and 39 HIV-infected patients, the variability of the erythromycin breath test (ERMBT) expressed as the CV was 24% versus 51% in HIV-infected patients [81]. In our own pharmacokinetic studies with PIs during controlled and standardized circumstances, we also observed significant inter-individual variability in PI metabolism and excretion. In four studies including between 6 to 9 HIV-infected patients or healthy volunteers, we observed a ratio ranging from 3.6 to 10.3 when the highest $C_{through}$ obtained in the study were divided by the lowest $C_{through}$ [III-V, VII].

CYP3A5, which resembles CYP3A4, might also contribute to the metabolism of PIs [82]. CYP3A5 is mainly present in the gastrointestinal system but at much lower levels than CYP3A4 [82-84]. Several CYP3A5 polymorphisms have been identified as a possible (pharmacogenetic) cause of inter-individual variation of e.g. saquinavir concentrations [85-87].

Nelfinavir is primarily metabolised by CYP2C19 but also CYP3A4 and to a lesser extent CYP2C9 and CYP2D6 [88]. CYP2C19 is responsible for the formation of the nelfinavir active metabolite, M8, which possesses *in vitro* antiretroviral activity comparable to that of nelfinavir [88, 89]. M8 is metabolised by CYP3A4 [43]. CYP2C19 polymorphisms have also been identified. Among the Asian population, 20% are poor metabolisers, compared with 2% of the Caucasian population, and consequently have very low concentrations of M8 (pharmacogenetic variation) [43, 89].

In studies with [^{14}C]radiolabelled PIs, 75% to 87% of a PI dose was recovered in faeces, either as unchanged drug or metabolite, suggesting that excretion is mainly biliary [37]. Renal excretion of unchanged drug or metabolite accounts for approximately 1-14% of a PI dose [37].

Apart from being substrates for CYP isozymes, the PIs are also inhibitors of CYP3A4, exerting mechanism-based inhibition, which involves inactivation of the enzyme by tightly and irreversible binding of reactive metabolites that are formed as a result of the oxidative metabolism [90-95]. In case of ritonavir, amprenavir, indinavir and nelfinavir, it has been possible to identify specific reactive metabolites which are probably responsible for the inactivation of CYP3A4 [95]. Some of the PIs, ritonavir, nelfinavir, amprenavir and lopinavir also have the ability to induce CYP3A4 [III, 96-98]. Ritonavir is also an inducer of CYP1A2 and CYP2C9 and the combination of lopinavir/ritonavir induces CYP2C19 [37, 99]. Induction of CYP3A4 by the PIs is caused by binding, thereby activation of the nuclear pregnane X receptor (PXR), which functions as a heterodimer with the nuclear retinoid X receptor (RXR). This heterodimer can bind to promoter regions of the *CYP3A* gene and regulate gene expression [100, 101].

P-glycoprotein

P-glycoprotein is an ATP-dependent drug-transporter located in the plasma membrane on the luminal (apical) side of various types of cells e.g. enterocytes, hepatocytes, renal tubular cells and the endothelial cells of the blood-testis and blood-brain barrier, but also CD4 cells [97, 102]. It is the multidrug resistance (MDR) gene (*MDR1*) which encodes for P-glycoprotein. P-glycoprotein serves as a protective mechanism (efflux pump) against various compounds which the human body is exposed to, thus promoting intestinal, hepatobiliary and renal excretion of foreign compounds and preventing exposure of the testis and brain (Figure 1) [57, 59, 65]. In *in vitro* studies, it has been shown that all the PIs are P-glycoprotein substrates but also P-glycoprotein inhibitors [94, 103-105]. Ritonavir is the most potent inhibitor, although ritonavir is only a moderate inhibitor compared to e.g. the P-glycoprotein inhibitor LY335979 [106-110]. Some studies have also shown that some of the PIs, e.g. ritonavir, nelfinavir, amprenavir, lopinavir and atazanavir, can induce P-glycoprotein *in vitro* [94, 97, 105, 111, 112]. In a small study (n=6), induction of P-glycoprotein and/or CYP3A4 beyond four weeks was linked with decreases in saquinavir concentrations, even after long-term (>12 weeks) therapy [113]. However, this has not been demonstrated in other long-term studies with saquinavir or other PIs (Table 2). Functional variants of P-glycoprotein have been identified and are caused by single nucleotide polymorphisms (SNP) in the *MDR1* gene [114]. The functional variants are distributed with varying population frequencies according to racial background and are another example of a genetic cause for pharmacokinetic variation [115].

The complex interaction between drug-transporters and drug-metabolising enzyme is not fully elucidated and the clinical implications even less. In a study by Fellay et al, the pharmacogenetics of CYP3A4/5, CYP2D6, CYP2C19 and P-glycoprotein were investigated in HIV-infected patients (n=123) receiving nelfinavir or the NNRTI efavirenz [116]. The main result was that patients with a MDR1 3435 TT genotype (low P-glycoprotein expression) had a greater rise in the CD4 cell count, compared with the MDR1 3435 CT and CC genotype, which corresponds with the hypothesis that low P-glycoprotein expression results in high intracellular concentrations [117, 118]. However, it was surprising that these patients had very low nelfinavir plasma concentrations [116]. In another study by Saitoh et al including HIV-infected children (n=71) receiving nelfinavir and efavirenz, the MDR1 3435 CT genotype (lower P-glycoprotein expression) was associated with higher nelfinavir plasma concentrations and a more rapid virological response (plasma HIV RNA <400 copies/ml) [119]. The results of this study are more in agreement with the hypothesis of the possible impact of P-glycoprotein.

The possible therapeutic implications of P-glycoprotein located in the blood-brain barrier and the effect on PI penetration into the central nervous system was investigated in the EuroSIDA cohort, including 9803 patients [59, 120]. It was hypothesised that the inci-

dence of the AIDS dementia complex (ADC), which is caused by HIV itself, could be affected by the choice of PI (only indinavir penetrates the blood-brain barrier in therapeutic concentrations). The study showed that although a significant overall decline in central nervous system disease, including ADC, could be demonstrated simultaneously with the introduction of HAART, the use of indinavir as compared with other PIs was not associated with a lower risk of developing central nervous system disease [120]. A study by Antinori et al confirmed that penetration into the central nervous system varied between PIs but also that the resistance patterns of HIV in the central nervous system and plasma were different [64]. The authors suggested that it could be useful to investigate the central nervous system to look for potential resistance in case of virological failure [64]. Although the results are not clear, problems with the development of different resistance patterns in the central nervous system might be accentuated in cases where PIs with poor penetration into the central nervous system are used as monotherapy for maintenance therapy, e.g. lopinavir or atazanavir [121, 122].

The considerable overlap in tissue distribution and substrate specificity between CYP3A and P-glycoprotein has made it complicated to evaluate the importance of P-glycoprotein alone [123]. Conclusive information on the significance of P-glycoprotein will only be obtained if selective P-glycoprotein and CYP3A inhibitors are developed for clinical use. Recently the investigational P-glycoprotein inhibitor tariquidar has been used to demonstrate that the erythromycin breath test is not only a measure of CYP3A activity but also P-glycoprotein function [124]. The role of other drug-transporters, such as the multidrug resistance-associated protein (MRP1) in PI transport and excretion has also been investigated but data are still limited. The possible clinical implications of e.g. MRP1 is uncertain, although it has been shown *in vitro* and *in vivo* to mediate PI efflux [117, 118, 125].

Intracellular concentrations versus plasma concentrations

All the PIs accumulate within PBMCs including CD4 cells, but with varying ratios compared to plasma. Intracellular/plasma AUC and C_{min} ratios *in vivo*: saquinavir AUC 4-17.6 and C_{min} 8.9 [126, 127]. Ritonavir AUC 1-4.6 and C_{min} 3.3 [41, 126, 127]. Indinavir AUC 0.3-0.5 and C_{min} 1 [126, 128]. Nelfinavir AUC 5.3-9 and C_{min} 3.6-5.4 [129, 130]. Amprenavir C_{min} 1.6-4.8 [131]. Lopinavir AUC 1.2 and C_{min} 1.4-3.2 [41, 132]. Atazanavir (co-administered with saquinavir and ritonavir) AUC 1.2 and C_{min} 2.1 [133]. Variations in ratios from different studies can be attributed to differences in methods for intracellular concentration measurement and co-administration of other PIs, especially ritonavir. Efflux/drug transporters might influence the ratio on an inter-individual basis (discussed earlier and below).

As the PIs exert their pharmacological effect intracellularly, the intracellular concentration of PIs has been regarded as an important parameter to investigate. Studies most often report PI intracellular concentrations as total intracellular concentrations but PIs are also bound to intracellular proteins, which further complicates the interpretation of the data [126].

Very high saquinavir intracellular/plasma AUC_{24} ratios (median 17.6) were demonstrated in HIV-infected patients receiving the saquinavir HGC in a study by Khoo et al [126]. This was a result of a low absolute plasma AUC_{24} (denominator). The intracellular AUC_{24} of the saquinavir HGC was comparable to the saquinavir SGC, but the corresponding plasma AUC_{24} was twice as high (median ratio 7.5). Saquinavir in combination with ritonavir resulted in 4- to 5-fold higher intracellular AUC_{24} but the plasma AUC_{24} was also very high (median ratio 4) [126]. High saquinavir intracellular/plasma AUC_{24} (3.3) and C_{24} (7.6) ratios were also reported in a study by Ford et al with once-a-day administration of saquinavir HGC and ritonavir [127]. In this study, a correlation between the intracellular and plasma AUC_{24} was demonstrated but also a longer intracellular $t_{½}$ (5.9 hours) compared with plasma $t_{½}$ (4.5 hours). No association between P-glycoprotein expression and intracellular concentrations was found, which was also demonstrated in an earlier study by the same group [118, 127]. However, in this study, an association between lower MRP1 expression and higher intracellular concentrations of saquinavir was seen [118]. The high saquinavir intracellular/plasma concentration ratios and an apparent longer intracellular $t_{½}$ *in vivo* compared with plasma have been associated with the saquinavir plasma concentration-efficacy discrepancy observed in the CHEESE study [134]. In this study 86% (19/22) of the patients had plasma HIV RNA <50 copies/ml after 48 weeks, although a low saquinavir C_{min} (<100 ng/ml) was demonstrated on at least one occasion in 77% of the patients (17/22).

Indinavir has the lowest intracellular/plasma ratios (lowest in combination with ritonavir), which is surprising considering that indinavir has the highest fraction of unbound drug [126]. The indinavir intracellular $t_{½}$ has also been shown to be longer (2.0 hours) than the plasma $t_{½}$ (1.2 hours) in a study by Hennessy et al [128]. In this study, no correlation was demonstrated between intracellular and plasma AUC_8 or $C_{through}$ but the study included only 10 patients [128].

In another study, by the same investigators, a good correlation was found between nelfinavir intracellular concentrations and plasma concentrations with a nelfinavir intracellular/plasma AUC_{12} and C_{12} ratio of 9.0 and 5.4 [129]. Intracellular concentrations were also positively correlated with P-glycoprotein function (assessed by rhodamine efflux), i.e. higher concentrations equals higher function, but not with P-glycoprotein expression on the cell surface [129]. This was also demonstrated by Ford et al, which suggests that P-glycoprotein function is perhaps concentration-dependent but also that the association between expression and function is not straightforward [130].

The lopinavir intracellular concentration and plasma concentration have been shown to correlate well, with an intracellular/plasma AUC_{12} ratio of 1.2 [41]. The corresponding intracellular/plasma AUC_{12} ratio of the co-administered ritonavir was 4.6. A study including HAART-experienced patients (n=38) by Breilh et al confirmed the correlation between lopinavir intracellular C_{min} and plasma C_{min} (ratio: 1.4-3.2) and hence the intracellular concentration did not add information in most cases. However, in some patients (n=8), a high intracellular C_{min} (>8000 ng/ml) predicted virological success (plasma HIV RNA <50 copies/ml at 6 month) despite relatively low plasma C_{min} (2500-4000 ng/ml) [132]. In this study, it was also shown that, although the correlation between intracellular concentrations and plasma concentrations persisted at month 1 and month 6, the ratio was not the same with intracellular concentrations decreasing over time [132].

To summarise, in most cases, PI intracellular concentrations correlate well with plasma concentrations, e.g. saquinavir, nelfinavir and lopinavir. However, data regarding indinavir are not persuasive. Ritonavir does not increase the intracellular/plasma ratio of saquinavir and it decreases the ratio of indinavir but total intracellular concentrations are still higher with ritonavir than without at standard doses [126]. So far, no studies have demonstrated a more clear association between PI intracellular concentrations and efficacy *in vivo* compared with plasma concentrations. In a minority of patients with a discrepancy between intracellular concentrations and plasma concentrations, it has been shown that the intracellular concentration in some cases can explain virological success despite low plasma concentrations.

Diurnal variation

Diurnal variation of drug concentrations has been reported for many drugs, e.g. digoxin, verapamil, terbutaline and diazepam. Variation is also known to occur for biological parameters (heart rate, blood pressure, renin, cortisol and insulin) [135]. Such variations may explain diurnal variation of drug concentrations, although the direct mechanism(s) has not been established.

Diurnal variation of PI plasma concentrations was first demon-

strated in a phase I study with ritonavir in HIV-infected patients by Hsu et al (1997) [96]. The study was a pharmacokinetic study and patients were confined to a research facility during the study. No co-medication was allowed since this could be a source of error, e.g. if a drug was dosed once-a-day concomitantly with the twice-a-day administration of ritonavir. Small changes in protein binding were observed which only partially accounted for the diurnal variation (morning $C_{through}$/evening $C_{through}$ ratio: 1.5, Table 1). It was believed that the plasma lipid composition, which changed during the day, had some effect on protein binding. In a population study by Baede-van Dijk et al, a nelfinavir concentration-time curve was constructed with 618 plasma samples from 355 patients receiving nelfinavir 1250 mg twice-a-day [43]. Data on co-medication was available and some of the patients received once-a-day administration of a drug, which could interact with nelfinavir (efavirenz, rifabutin, omeprazole). The curve demonstrated that the morning $C_{through}$ was 2.5-fold higher than the evening $C_{through}$ but the observation was not discussed any further. Diurnal variation has also been observed in pharmacokinetic studies with saquinavir, indinavir and amprenavir (Table 1) [I]. In 25 out of 29 patients, the morning $C_{through}$ was higher than the evening $C_{through}$ (ratio range: 0.8-4.1). It was suggested that dosing intervals could be changed to less than 12 hours between the morning and evening dose to obtain more balanced PI concentrations. Recently, it has also been shown that lopinavir concentrations are displaying diurnal variation (Table 1).

Any clinical implications of diurnal variation have not been established, but it has some important perspectives. As demonstrated in Figure 2, in a patient displaying significant diurnal variation in lopinavir concentrations, the concentration in the morning would be considered as low (1849 ng/ml, reference: 5500 ng/ml) whereas the evening concentration is extremely low (108 ng/ml) [136]. It is mainly the $t_{½}$ that is affected, from 5.9 hours to 1.6 hours, suggesting that hepatic metabolism is the key factor in diurnal variation. Pharmacokinetic data are collected as drugs are investigated in phase I and II trials. Data are usually derived from pharmacokinetic studies with multiple blood sampling during a dosing interval. The C_{min} or $C_{through}$ data are usually obtained 8-12 hours after a morning dose of the drug. These values are reported as the C_{min} or $C_{through}$. Outside pharmacokinetic studies blood sampling is typically performed in the morning 8-12 hours after the last dose and discrepancies could arise as the morning $C_{through}$ might be several fold higher than the evening $C_{through}$. These variations should be considered when evaluating PI $C_{through}$ results from pharmacokinetic studies (often evening $C_{through}$) or from patients attending an outpatient clinic (often morning $C_{through}$), e.g. study no. 6 (220 ng/ml versus 434 ng/ml) and study no. 4 versus no. 13 (680 ng/ml versus 1553 ng/ml) in Table 3 [IV, VIII, 137].

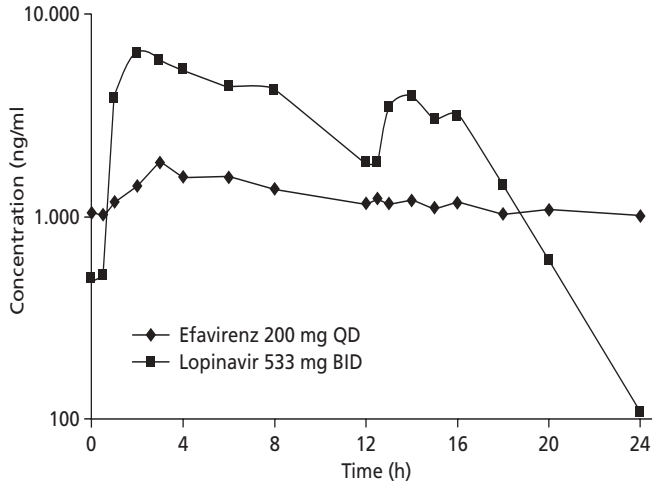

Figure 2. 24-h pharmacokinetic profile of efavirenz 200 mg QD and lopinavir/ritonavir 533/133 mg BID (0 h and 24 h correspond to 08.00 p.m.). Mathiesen et al. Scandinavian Journal of Infectious Diseases 2006 [136]. QD: once-a-day. BID: twice-a-day.

PROTEASE INHIBITORS AND DRUG-DRUG INTERACTIONS

As a consequence of the metabolism of PIs by the Cytochrome P450 system and excretion by drug-transporters such as P-glycoprotein, the pharmacokinetic drug-drug interaction potential with PIs is considerable. PI drug-drug interactions can be used to improve PI efficacy (pharmacokinetic enhancement) but may also cause adverse effects.

Pharmacokinetic enhancement

Ritonavir

By using ritonavir in low non-therapeutic doses (low-dose, 50-200 mg once or twice-a-day), it is possible to increase bioavailability and decrease clearance of other co-administered PIs, primarily by inhibition of intestinal and hepatic CYP3A4 and possibly P-glycoprotein (pharmacokinetic enhancement or boosting, Table 3) [VII, VIII, 138]. Pharmacokinetic enhancement can increase PI concentrations despite dose reductions, e.g. saquinavir 1200 mg three-times-a-day compared with squinavir/ritonavir 1000/100 mg twice-a-day and amprenavir 1200 mg twice-a-day compared with fosamprenavir/ritonavir 700/100 mg twice-a-day (Appendix 1). The indinavir dose can be reduced to a third from 800 mg three-times-a-day to 400 mg twice-a-day in combination with ritonavir 100 mg without reducing the indinavir $C_{through}$ (study no. 14, Table 3) [IV, 139, 140]. Furthermore, ritonavir co-administration eliminates the reduced absorption of indinavir when taken with food, which means that indinavir/ritonavir can be administered without special food requirements (study no. 18 and 19, Table 3) [141-143]. In a single dose study including healthy volunteers receiving a lopinavir dose of 400 mg, co-administration of 50 mg of ritonavir resulted in a 77-fold increase of the lopinavir AUC_{24} and consequently lopinavir has been co-formulated with ritonavir by the manufacturer [138]. Atazanavir, which is dosed once-a-day, is only licensed by the EMEA with ritonavir co-administration [37]. It is also possible to apply pharmacokinetic enhancement on two PIs, e.g. lopinavir and saquinavir, simultaneously (double boosting) [144]. Nelfinavir is primarily metabolised by CYP2C19, which is not significantly inhibited by ritonavir, and secondly CYP3A4. Therefore, pharmacokinetic enhancement with ritonavir results in relatively small increases in nelfinavir $C_{through}$ (51%) whereas the $C_{through}$ of the nelfinavir active metabolite M8, which is metabolised by CYP3A4, increases more than 5-fold [VII]. The result is that the concentration of nelfinavir + M8 $C_{through}$ is more than doubled [VII]. Pharmacokinetic enhancement with large increases in PI plasma concentrations has been associated with more toxicity for some of the PIs, saquinavir, indinavir (study no. 15, Table 3) and amprenavir [VIII, 145]. Apparently, pharmacokinetic enhancement of nelfinavir is not associated with more toxicity, although studies are scarce [VII, 146]. Pharmacokinetic enhancement can also be used to reduce toxicity without reducing the $C_{through}$. If indinavir 800 mg three-times-a-day is changed to indinavir/ritonavir 400/100 mg twice-a-day, the C_{max} is reduced considerably without reducing the $C_{through}$ [IV, 147]. The indinavir C_{max} has been shown to be associated with urological toxicity (discussed below) [137].

Delavirdine

Delavirdine is a NNRTI. It is not licensed in Europe. *In vitro* studies have shown that delavirdine is metabolised by CYP3A [148]. It is also a strong inhibitor of CYP3A, which has been shown to be mechanism-based [149, 150]. Delavirdine is probably also an inhibitor of P-glycoprotein [151]. *In vivo* studies have confirmed the CYP3A inhibitory potential of delavirdine although pharmacokinetic enhancement of co-administered PIs have been moderate compared with ritonavir [III, 152-154]. Combinations with NNRTIs and PIs are appealing from a doctor-patient point of view, if the combination permits reduced doses but maintains antiretroviral effect from

Table 3. Pharmacokinetics from studies investigating different doses of indinavir with and without ritonavir pharmacokinetic enhancement.

No.	Indinavir (mg)	Ritonavir (mg)	Type of study	Subjects	N	$C_{through}$ (ng/ml)		References
1)	800×3		Efficacy	HIV-infected patients	65	134 (median)	34-669 (range)	Burger et al [25]
2)	800×3		Pharmacokinetic	Healthy volunteers	8	150 (mean)	±80 (SD)	Hsu et al [141]
3)	800×3		Pharmacokinetic	HIV-infected patients	10	177 (median)	81-496 (range)	Boffito et al [219]
4)	800×3		Pharmacokinetic	HIV-infected patients	19	130 (median)	90-270 (IQR)	Burger et al [137]
5)	800×3		Pharmacokinetic	HIV-infected patients	12	250 (G mean)	185-337 (90% CI)	Rhame et al [220]
6)	400×2	100×2	Pharmacokinetic	HIV-infected patients	9	220 (median)	102-364 (range)	Justesen et al (IV)
7)	400×2	100×2	Efficacy and toxicity	HIV-infected patients	34	500 (median)	5-8100 (range)	Duvivier et al [139]
8)	400×2	100×2	Pharmacokinetic	HIV-infected patients	19	170 (median)	120-300 (IQR)	Boyd et al [140]
2)	400×2	300×2	Pharmacokinetic	Healthy volunteers	7	260 (mean)	±80 (SD)	Hsu et al [141]
	400×2	400×2			8	400 (mean)	±180 (SD)	
9)	400×2	400×2	Efficacy and toxicity	HIV-infected patients	32	450 (median)		Burger et al [181]
3)	400×2	400×2	Pharmacokinetic	HIV-infected patients	5	436 (median)	223-4103 (range)	Boffito et al [219]
10)	400×2	400×2	Efficacy and toxicity	HIV-infected patients	22	621 (median)	114-1561 (range)	Acosta et al [221]
2)	600×2	200×2	Pharmacokinetic	Healthy volunteers	8	430 (mean)	±140 (SD)	Hsu et al [141]
	600×2	300×2			8	550 (mean)	±260 (SD)	
5)	667×2	100×2	Pharmacokinetic	HIV-infected patients	12	1511 (G mean)	1119-2039 (90% CI)	Rhame et al [220]
11)	800×2	100×2	Pharmacokinetic	HIV-infected patients	6	990 (mean)	580-1400 (95% CI)	van Heeswijk et al [222]
9)	800×2	100×2	Efficacy and toxicity	HIV-infected patients	100	770 (median)		Burger et al [181]
3)	800×2	100×2	Pharmacokinetic	HIV-infected patients	5	276 (median)	250-1734 (range)	Boffito et al [219]
4)	800×2	100×2	Pharmacokinetic	HIV-infected patients	17	680 (median)	430-770 (IQR)	Burger et al [137]
12)	800×2	100×2	Pharmacokinetic	HIV-infected patients	12	558 (median)	159-2453 (range)	Justesen et al (V)
13)	800×2	100×2	Efficacy and toxicity	HIV-infected patients	83	1553 (median)	952-3362 (IQR)	Justesen et al (VIII)
10)	800×2	200×2	Efficacy and toxicity	HIV-infected patients	22	702 (median)	80-2919 (range)	Acosta et al [221]
11)	1200×2		Pharmacokinetic	HIV-infected patients	6	210 (mean)	0-670 (95% CI)	van Heeswijk et al [222]
	1200×2	100×2			2	1730 (mean)	930 and 2530	
	Comparative studies							
14)	800×3		Efficacy	HIV-infected patients	20	194 (median)	35-922 (range)	Ghosn et al [147]
	400×2	100×2				475 (median)	7-2462 (range)	
15)	800×3		Efficacy	HIV-infected patients	10	130 (median)		Arnaiz et al [223]
	800×2	100×2				500 (median)		
16)	400×2	100×2	Pharmacokinetic	Healthy volunteers	15	190 (G mean)	80-360 (range)	Wasmuth et al [186]
	600×2	100×2				490 (G mean)	160-1820 (range)	
17)	400×2	100×2	Pharmacokinetic	HIV-infected patients	11	170 (median)	100-390 (range)	Cressey et al [187]
	600×2	100×2				410 (median)	120-770 (range)	
	Special situations							
18)	400×2 Low fat meal	400×2	Pharmacokinetic	Healthy volunteers	10	1308 (G mean)	334-3390 (range)	Saah et al [142]
	High fat meal					1161 (G mean)	315-2874 (range)	
	800×2 Low fat meal	100×2			10	1396 (G mean)	816-4701 (range)	
	High fat meal					1371 (G mean)	578-5934 (range)	
	800×2 Low fat meal	200×2			8	3119 (G mean)	1019-6039 (range)	
	High fat meal					3281 (G mean)	919-6791 (range)	
	800×2 Low fat meal	400×2			9	3105 (G mean)	1754-8687 (range)	
	High fat meal					3392 (G mean)	1321-5536 (range)	
19)	800×2 Without food	100×2	Pharmacokinetic	HIV-infected patients	9	450 (G mean)	160-1400 (range)	Aarnoutse et al [143]
	With food					440 (G mean)	180-1200 (range)	

G mean: geometric mean. SD: standard deviation. IQR: interquartile range. CI: confidence interval.

both drugs, as opposed to low-dose ritonavir. In a study with indinavir/delavirdine 600/400 mg three-times-a-day, the mean indinavir $C_{through}$ increased more than 5-fold compared with indinavir 800 mg three-times-a-day [153]. A nearly 2-fold mean ritonavir $C_{through}$ increase was seen when ritonavir 600 mg twice-a-day was co-administered with delavirdine 400 mg three-times-a-day [154]. In our own study, pharmacokinetic enhancement of amprenavir 600 mg with delavirdine 600 mg twice-a-day resulted in a more than 2-fold increase of median amprenavir $C_{through}$, which was comparable to administration of amprenavir 1200 mg twice-a-day alone [III]. However, in this study a considerable and unfavourable decrease of 88% in the median delavirdine $C_{through}$ was also seen, which was believed to be caused by amprenavir CYP3A4 induction [III]. In a follow-up study, it was investigated if the inducing effect of amprenavir could be compensated for by increasing the dose of delavirdine (800-1000 mg) [VI]. This was partly achieved as the delavirdine $C_{through}$ increased nearly 5-fold with only a 67% increase of delavirdine dose [VI]. The amprenavir $C_{through}$ increased as well, despite a dose reduction to 450 mg when dosed with 1000 mg of delavirdine [VI].

Adverse PI drug-drug interactions

The consequence of adverse PI drug-drug interactions can be divided into four groups; decreased efficacy or increased toxicity associated with either the PI or co-administered drug. Drug-drug interactions causing increased PI toxicity have been discussed earlier.

Decreased efficacy of PI

Rifampicin is an important drug in the treatment of tuberculosis, which is a common complication among HIV-infected patients [155]. Rifampicin is a very strong inducer of CYP3A4. The mechanism is binding followed by activation of the nuclear pregnane X receptor which is important for the regulation of *CYP3A* gene expression. Concentrations of all PIs are significantly reduced when co-administered with rifampicin (Table 4). This may lead to virological failure. Therefore, treatment of tuberculosis in patients receiving PI containing HAART constitutes a particular problem. In 1999, a study in two HIV-infected patients receiving saquinavir/ritonavir 400/400 mg and 1000/100 mg twice-a-day, demonstrated that saquinavir concentrations were apparently not affected by rifampicin co-administration [156]. The authors hypothesised that the inhibitory effect of ritonavir compensated for the inducing effect of rifampicin. Subsequently, the hypothesis has been tested in other studies with saquinavir (Table 4) without convincing results. In 2005, the manufacturer of saquinavir had to issue a warning because of severe hepatocellular toxicity in healthy volunteers after only a few days of saquinavir, ritonavir and rifampicin administration

Table 4. Pharmacokinetic interactions between rifampicin and protease inhibitors.

Protease inhibitor	Rifampicin dose	Subjects	N	$C_{through}$ (ng/ml)		Comment	References
Saquinavir HGC/ ritonavir 1000/100 mg ×2	600 mg ×1 for 1-5 days	Healthy volunteers	17	-	-	Severe hepatocellular toxicity in 11/17. Combination is contraindicated.	EMEA [37]
Saquinavir/ ritonavir 400/400 mg ×2	600 mg ×1 for 4 weeks	HIV-infected patients	1	>500, 8 hours after administration	No comparison	Observational study.	Veldkamp et al [156]
Saquinavir/ ritonavir 1000/100 mg ×2	450 mg ×1 for 4 weeks		1				
Saquinavir SGC 1200 mg ×3	600 mg ×1 for 14 days	HIV-infected patients	11	62 to 27 (G mean)	56% decrease	Interaction study. Combination is contraindicated.	Grub et al [224]
Saquinavir SGC/ ritonavir 1600/200 mg ×1	600 mg ×1 for >4 weeks	HIV-infected patients	17		44% decrease	Median saquinavir $C_{through}$ (from 28 patients during treatment with rifampicin): 80 ng/ml.	Ribera et al [225]
Saquinavir SGC/ ritonavir 1600/200 mg ×1	600 mg ×1 for 8 weeks	HIV-infected patients	15	140 to 60 (median)	57% decrease	Saquinavir $C_{through}$ after and during treatment with rifampicin.	Ribera et al [226]
Ritonavir 600 mg ×2	600 mg ×1 for 8 weeks	HIV-infected patients	8	2220 (median), 350-9670 (range)	No comparison	Observational study.	Moreno et al [158]
Indinavir		HIV-infected patients			90% decrease	No data. Combination is contraindicated.	EMEA [37]
Indinavir/ ritonavir 800/100 mg ×2	300 mg ×1 for 4 days	HIV-infected patients	6	837 to 112 (median)	87% decrease	Interaction study. Combination is contraindicated.	Justesen et al [V]
Nelfinavir						82% decrease in AUC. No data. Combination is contraindicated.	EMEA [37]
Amprenavir 1200 mg ×2	600 mg ×1 for 18 days	Healthy volunteers	11		92% decrease	Interaction study. Combination is contraindicated.	Polk et al [227]
Lopinavir/ ritonavir 400/100 mg ×2		Healthy volunteers			99% decrease	No data. Combination is contraindicated.	la Porte et al [157]
Lopinavir/ ritonavir 800/200 mg ×2	600 mg ×1 for 14 days	Healthy volunteers	10	6500 to 5100 (G mean)	57% decrease	The two regimens were compared to lopinavir/ritonavir 400/100 mg.	
Lopinavir/ ritonavir 400/400 mg ×2			9	5200 to 5900 (G mean)	3% increase		
Atazanavir/ ritonavir 300/100 mg ×1	600 mg ×1 for 10 days	Healthy volunteers	16	707 to 18 (G mean)	97% decrease	The three regimens were compared to atazanavir/ritonavir 300/100 mg x 1. Combination is contraindicated.	Burger et al [228]
Atazanavir/ ritonavir 300/200 mg ×1			17	707 to 43 (G mean)	94% decrease		
Atazanavir/ ritonavir 400/200 mg ×1			14	707 to 53 (G mean)	93% decrease		

HGC: hard-gel capsule. SGC: soft-gel capsule. G mean: geometric mean.

(Table 4). No concentration data were provided but toxicity might have been caused by increasing rifampicin concentrations, which have been observed in other interaction studies with ritonavir and atazanavir (Table 4). Interaction studies with indinavir and lopinavir have also been conducted (Table 4). In a small study in HIV-infected patients (n=6), the median indinavir $C_{through}$ was reduced by 87% after only 4 days of low-dose (300 mg) rifampicin administration despite co-administration of ritonavir [V]. In another study with lopinavir, including healthy volunteers, it was shown that by increasing the dose of lopinavir and/or ritonavir (lopinavir/ritonavir 400/400 mg or 800/200 mg), it was possible to compensate for the inducing effect of rifampicin and achieve lopinavir concentrations similar to concentrations achieved with the standard dose (Table 4) [157]. However, short-term as well as long-term toxicity could very well be treatment limiting factors, as 12 of 32 healthy volunteers dropped out of the study because of adverse events or laboratory abnormalities. The concomitant use of ritonavir and rifampicin has been considered as feasible by some. However, only one study addresses this issue (Table 4) [158]. Eighteen patients receiving ritonavir 600 mg twice-a-day and rifampicin were included in the study but 10 patients left the study before week 8 (6 cases of toxicity). Ritonavir concentrations were available for the remaining 8 patients with a median $C_{through}$ of 2220 ng/ml, which means that half of the patients had concentrations below what is considered as therapeutic (2100 ng/ml) [21].

Efavirenz is also a strong inducer of hepatic CYP3A4, but in contrast to rifampicin it has been possible to compensate for the inducing effect [159, 160]. In case of lopinavir, a modest increased dose of lopinavir/ritonavir 533/133 mg twice-a-day in combination with efavirenz results in concentrations comparable to lopinavir/ritona-

vir 400/100 mg [161]. However, caution should be exercised as drug-drug interactions can be unpredictable. This is illustrated in Figure 2. The patient received a low dose of efavirenz (200 mg versus standard dose 600 mg) in combination with an increased dose of lopinavir but achieved only very low concentrations of lopinavir, probably because of extensive hepatic CYP3A4 induction [136]. Other examples of CYP3A4 inducers, where clinically significant drug-drug interactions have been reported, include St John's wort (*Hypericum perforatum*), a herbal remedy, which is believed to relieve symptoms of mild depression [162]. Drug-drug interactions, which do not include the cytochrome P450 system, have been reported with atazanavir. The bioavailability of atazanavir is pH-dependent and significant reductions (79%) in atazanavir C_{min} were demonstrated with omeprazole co-administration [163]. Co-administration of the nucleotide reverse transcriptase inhibitor (NtRTI) tenofovir has also been shown to decrease the atazanavir AUC_{24} by 25% [164]. The mechanism of this drug-drug interaction is unknown.

Decreased efficacy of co-administered drug

The PIs amprenavir, lopinavir and nelfinavir are inducers of the cytochrome P450 system which affects the metabolism of other drugs. This is a potential problem and might result in decreased efficacy of a co-administered drug, although not many clinically relevant interactions have been reported. Reductions in delavirdine $C_{through}$ when co-administered with amprenavir have been discussed earlier [III]. Ritonavir is also an inducer of the cytochrome P450 system but induction is compensated by the inhibitory effect of ritonavir and the net result is inhibition. However, other metabolic pathways, such as glucuronidation, are also induced by ritonavir. This is probably the reason why ritonavir has been shown to decrease ethinylestradiol AUC by 40% which could affect the efficacy of oral contraceptives [165].

Increased toxicity of co-administered drug

All of the PIs are CYP3A4 inhibitors, which could result in increased toxicity of a co-administered drug. Some drugs with narrow therapeutic indexes are contraindicated in combination with PIs in general. The antihistamines, astemizole and terfenadine, the gastrointestinal agent cisapride and the antipsychotic drug pimozide are all contraindicated in combination with PIs because of CYP3A4 inhibition. Inhibition increases the concentrations of the co-administered drug and the risk of cardiac arrhythmias including ventricular tachycardia, ventricular fibrillation, torsades de pointes and QT prolongation. For some of the statins (HMG-CoA reductase inhibitors), concentrations are markedly increased by PIs, especially ritonavir, e.g. lovastatin and simvastatin [166]. Statins can cause serious toxicity, including rhabadomyolysis, and are often administered in HIV-infected patients because of lipid disturbances [166].

There are numerous published reviews about PI drug-drug interactions with advice on how to manage problems, but reviews are quickly outdated [167-169]. The use of updated interaction databases which can be accessed via the internet have made the management of PI drug-drug interactions easier [42, 170]

In summary, several factors may cause inter-individual and intra-individual variations of PI concentrations; pharmacokinetic, including pharmacogenetic (CYP3A5, CYP2C19 and P-glycoprotein) and drug-drug interactions. However, the question is to what extent this has clinical implications with regard to PI efficacy or toxicity in HIV-infected patients receiving PI therapy?

IMPORTANCE OF PROTEASE INHIBITOR CONCENTRATIONS

EFFICACY

Concentration-efficacy associations have been established for all the PIs in PI-naïve patients harbouring wild type HIV without PI-associated resistance mutations. A concentration-efficacy association has also been established for some PIs in PI-experienced patients harbouring HIV with PI-associated resistance mutations and varying degrees of resistance/reduced susceptibility. However, quite different approaches have been used in the studies investigating PI concentration-efficacy associations.

Saquinavir

A concentration-efficacy study with different doses of saquinavir SGC (400-1200 mg) and saquinavir HGC (600 mg) administered three-times-a-day as monotherapy was completed by Gieschke et al [20]. By investigating six different mathematical models and hereafter applying two different quantitative model selection criteria (Schwartz and Akaike), a 2-parameter E_{max} model was shown to best predict the concentration-efficacy relationship (Parameter 1: E_{max} and parameter 2: EC_{50}). With this model, an EC_{50} of 50 ng/ml was predicted with a CV of 40% [20]. In ACTG study 359 with indinavir-experienced patients receiving saquinavir and two or three other antiretroviral drugs (ritonavir, nelfinavir, delavirdine or adefovir), it was shown that higher saquinavir $C_{through}$ were associated with a greater likelihood of a plasma HIV RNA ≤500 copies/ml after 16 weeks of treatment, median saquinavir $C_{through}$: 230 ng/ml versus 130 ng/ml. In patients with a baseline plasma HIV RNA ≥20000 copies/ml a saquinavir $C_{through}$ ≥100 ng/ml seemed to discriminate between patients with and without a plasma HIV RNA ≤500 copies/ml, 27% (n=78) versus 0% (n=36) [171]. This limit (and the number of saquinavir-associated resistance mutations) had also proven useful in a study by Valer et al to independently predict virological response (plasma HIV RNA <50 copies/ml or a ≥1 $\log_{10}$ decrease) after 24 weeks of treatment in a subset of PI-experienced patients with plasma HIV RNA >1000 copies/ml (n=73) [172]. In contrast, no concentration-efficacy association could be identified in the MaxCmin trials in a heterogeneous population of patients (PI-naïve and experienced) receiving saquinavir/ritonavir 1000/100 mg twice-a-day (n=130) [VIII]. The median saquinavir C_{min} in these patients was 1036 ng/ml with a total of 17 virological failures, 10 below and 7 above the median saquinavir C_{min} [VIII]. The results are not directly in conflict with the 100 ng/ml limit as no patients in the MaxCmin trials were below this limit; no virological failures were to be expected on the basis of too low saquinavir concentrations. Therefore, the results imply that other factors, such as adherence, might have caused virological failure.

Ritonavir

In *in vitro* studies with ritonavir, an EC_{50} (or IC_{50}) of approximately 0.009 to 0.046 μM in different strains of wild type HIV-1 was estimated with the EC_{90} value 2- to 3-fold higher in a medium of 10% foetal bovine serum [21, 173]. By testing in an *in vitro* system with progressively higher levels of human serum, the EC_{90} increased and appeared to be about 3 μM corresponding to 2100 ng/ml (ritonavir protein binding: 99%) which proved to be associated with efficacy in one of the first clinical studies with ritonavir, Danner et al (1995) [21].

Indinavir

In a study by Burger et al investigating HIV-infected patients (n=65) who started indinavir 800 mg three-times-a-day in combination with nucleoside analogues, it was shown that low plasma concentrations of indinavir was an independent predictor of virological treatment failure (study no. 1, Table 3) [25]. The patient indinavir concentrations were compared with a mean concentration-time curve derived from a control group (n=14) receiving the same regimen and a concentration ratio was calculated [25]. The C_{min} from the control group mean concentration-time curve was 140 ng/ml [25]. It was concluded that patients should have an indinavir concentration ratio above 0.70 corresponding to an indinavir C_{min} of 100 ng/ml to optimise therapy [25]. In a later study conducted by the same investigator with patients receiving indinavir/ritonavir

800/100 mg twice-a-day (n=17) or indinavir 800 mg three-times-a-day (n=19), this limit was confirmed in the group of patients receiving the three-times-a-day regimen but surprisingly the indinavir C_{min} in the other group was estimated to be 250 ng/ml for optimal virological efficacy with no apparent explanation. However, as has been discussed earlier, the intracellular/plasma concentration ratio is lower when indinavir is administered in combination with ritonavir, which might explain the difference [137]. In the study, a receiver operating characteristic (ROC) curve was constructed to identify possible concentration-efficacy associations, and although numbers were small a strong association was demonstrated with the 100 ng/ml and the 250 ng/ml limits. In the MaxCmin trials including patients (n=83) receiving indinavir/ritonavir 800/100 mg twice-a-day, no concentration-efficacy association could be established with regard to virological failure [VIII]. In the indinavir arm, the median indinavir C_{min} was very high, 1553 ng/ml at week 4, with no patients below 100 ng/ml. Four patients were below 250 ng/ml but this data was not published [VIII]. There were a total of 10 virological failures over 48 weeks, five below and five above the median, also indicating that other factors than the PI C_{min} are important for treatment failure [VIII]. Efficacy is most often in terms of suppression of viral replication or virological failure but the indinavir $C_{through}$ has also been associated with CD4 cell count response, e.g. Fletcher et al and the MaxCmin trials, although other studies have shown that the indinavir C_{max} is a better correlate [VIII, 174, 175]. Anderson et al reported that a C_{max} >7000 ng/ml was associated with greater increases in CD4 cell count [175].

Nelfinavir

A concentration-efficacy association has been demonstrated by Burger et al in a group of treatment-naïve patients (n=48) [26, 176]. As part of the ATHENA cohort study, nelfinavir concentrations were monitored and compared with a population concentration-time curve [26, 176]. Virological failure was defined as "no response" (detectable plasma HIV RNA after 6 months of treatment) or "relapse" (detectable plasma HIV RNA after being undetectable or an increase in plasma HIV RNA >1 $\log_{10}$) [26, 176]. The ratios in patients with and without virological failure were 0.77 and 0.99. By constructing a ROC curve, a ratio threshold of 0.9 was identified, corresponding to a nelfinavir minimum morning $C_{through}$ of 1440 ng/ml and a minimum evening $C_{through}$ of 770 ng/ml (diurnal variation) to optimise therapy [26, 176]. In another study including both PI-naïve and experienced patients (n=154), a C_{min} <1000 ng/ml was associated with virological failure in a multivariate analysis (as was the M36I mutation in the protease gene) [177]. In patients without nelfinavir-associated resistance mutations, the efficacy threshold was 800 ng/ml.

Amprenavir

In a pharmacokinetic and pharmacodynamic study of amprenavir in PI-naïve patients, a simple sigmoid E_{max} model was used to estimate the $C_{through}$ that yielded 50% to 99% of the maximum antiviral effect (Figure 3) [39]. The EC_{50}, EC_{90} and EC_{99} were 87 ng/ml, 228 ng/ml and 658 ng/ml, respectively. In a study with PI-experienced but amprenavir-naïve patients (n=49) experiencing virological failure, it was shown that treatment with amprenavir/ritonavir 600/100 mg reduced plasma HIV RNA with a median 1.32 $\log_{10}$. Response was significantly reduced in patients with six or more PI-associated resistance mutations but a C_{min} ≥1250 ng/ml proved to be efficacious in patients with up to five mutations [178].

Lopinavir

Molla et al examined the antiretroviral activity of lopinavir against different strains of wild type HIV in MT4 cells in the presence of 50% human serum [69]. In the study, an EC_{50} of approximately 70 ng/ml (31-82 ng/ml depending on the wild type HIV) was determined [69]. In a following clinical study with extensively pretreated

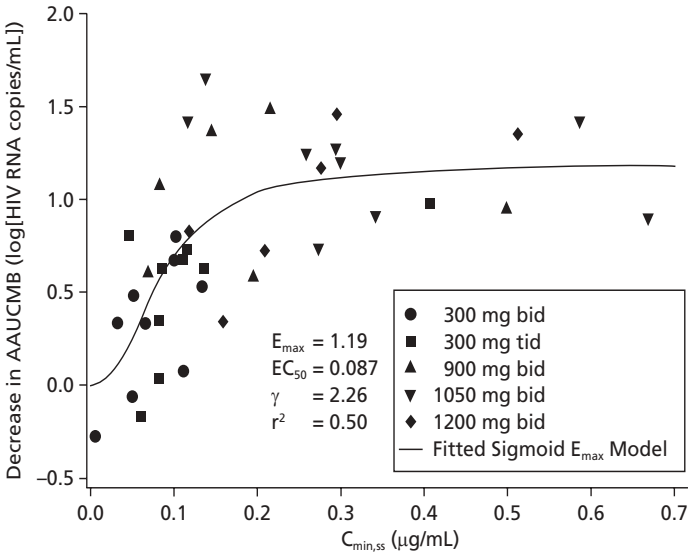

Figure 3. Fitted curve of amprenavir $C_{min,ss}$ versus decrease in AAUCMB for plasma HIV RNA using the sigmoid E_{max} model. For the model, estimated E_{max} = 1.19 (95% CI, 0.88 to 1.5) $\log_{10}$ copies/ml, EC_{50} = 0.087 (95% CI, 0.053 to 0.12) μg/ml, γ = 2.26 (95% CI, 0.14 to 4.4), r^2 = 0.50, and P < 0.0001. The %CVs for the estimated E_{max}, EC_{50}, and γ were 12.9%, 18.9%, and 46.4%, respectively. Sadler et al. Antimicrobial Agents and Chemotherapy 2001 [39]. Reproduced with permission from the authors and the Journals Department of the American Society for Microbiology. AAUCMB: time-weighted average decrease in $\log_{10}$ HIV RNA from baseline. γ: unitless shape parameter for sigmoid models. r^2: coefficient of determination.

patients (n=51), the IQ (inhibitory quotient, discussed below), which is the ratio of the PI $C_{through}$ to the IC_{50} of the HIV phenotype in question, was identified as a predictor of virological response [78, 161]. In patients (n=16) with an IQ >15, the virological response (plasma HIV RNA <400 copies/ml) was 100% after 24 weeks of treatment which, in patients harbouring wild type HIV, corresponds to a lopinavir C_{min} corrected for protein binding of 1000 ng/ml (≈15 ×70 ng/ml). In two other studies in experienced, lopinavir-naïve patients with 1 to 8 lopinavir-associated resistance mutations (n=38) and heavily pretreated patients (virological failure with other PIs, n=35), a concentration of 4000 ng/ml and ≥5700 ng/ml, respectively, was defined as the discriminating threshold for virological success or as a independent predictor of virological response (plasma HIV RNA <50 copies/ml) [132, 179]. In the MaxCmin trials, the median lopinavir C_{min} was 5131 ng/ml at week 4 (n=70, five patients had lopinavir C_{min} below 1000 ng/ml) [VIII]. No concentration-efficacy association could be demonstrated but out of nine virological failures, five, of which four were experienced patients, had lopinavir concentrations in the lower concentration quartile (C_{min} <3505 ng/ml) [VIII]. The results, however, did not reach statistical significance.

Atazanavir

Conference proceedings have described an association between the atazanavir $C_{through}$ and efficacy in a study of patients from an atazanavir-expanded access programme (n=51) [180]. Overall virological response (plasma HIV RNA <50 copies/ml) after 24 weeks was observed in 58%, 75% and 100% with atazanavir $C_{through}$s of <150 ng/ml, 150-850 ng/ml and >850 ng/ml, respectively. Study population data were limited but the median number of PI-associated resistance mutations (No. PAMs) was 2 [180]. An efficacy threshold >150 ng/ml was suggested [180].

TOXICITY

Although treatment with PIs has been related to numerous kinds of side effects, only a few have been directly associated with PI concentrations. Data have not always been conclusive and some studies are

conflicting. In studies where associations have been established, differences between the studies have made it difficult to establish guidelines or toxicity limits. This is most likely because the development of toxicity is a mixture of host susceptibility and PI concentrations.

Saquinavir

In a study with two high-dose saquinavir regimens (3600 mg and 7200 mg a day) without ritonavir, there were more adverse events, most commonly gastrointestinal, in the 7200 mg a day arm corresponding to higher median C_{min}, 39 ng/m versus 152 ng/ml. However, the C_{min} was very low compared to e.g. data from the MaxCmin trials (saquinavir with ritonavir), with a median saquinavir C_{min} of 1036 ng/ml [VIII, 18]. In the MaxCmin trials, it was demonstrated that a saquinavir C_{min} >2000 ng/ml was associated with an increased risk of gastrointestinal grade 3/4 adverse events but numbers were small [VIII]. The mechanisms behind the gastrointestinal adverse events in the two studies may be quite different as a systemic effect does not seem likely. In the first study toxicity could be related to the saquinavir dosage and corresponding high luminal concentrations whereas toxicity in the second study (MaxCmin) could reflect increased absorption of saquinavir in some patients and accordingly increased local exposure of drug when transported across the intestinal epithelium.

Ritonavir

Ritonavir is currently (2007) most often administered as a low-dose (100-200 mg) pharmacokinetic enhancer, which is probably only causing low-grade adverse events [145]. However, if ritonavir is dosed at 600 mg twice-a-day, adverse events are registered in many patients, e.g. 100% (n=15) in a phase I/II study during the first four weeks of treatment [21]. In a study comparing patients with (n=11) and without (n=10) ritonavir-related neurological or gastrointestinal toxicity, the median C_{max} and C_{min} were considerably higher in patients with toxicity, 26700 ng/ml and 12600 ng/ml versus 16200 ng/ml and 7500 ng/ml, but the study was too small to provide any guidelines or define a toxicity limit [22].

Indinavir

Indinavir-associated urological toxicity (haematuria, flank pain, crystalluria, nephrolithiasis, elevation of creatinine) have been reported in almost all clinical studies with indinavir and many have found associations between indinavir concentrations and urological toxicity [4, 23, 24, 137, 181-183]. In a case series study (n=15) by Dieleman et al, patients receiving indinavir 800 mg three-times-a-day with urological complaints (renal colic, flank pain or haematuria) were selected for indinavir concentration measurements. The concentrations were compared with a mean concentration-time curve derived from a control group (n=14) receiving the same regimen but without urological complaints [24]. Fourteen of the patients with urological complaints had a concentration above the mean and of these, 12 had a concentration above the upper 95% confidence limit. No pharmacokinetic data were supplied from the mean concentration-time curve or from the case series patients as all the data were reported as concentration ratios to the control group mean concentration. Consequently, no specific guidelines or toxicity limits could be established. In a retrospective study by Solas et al with patients (n=63) receiving indinavir/ritonavir 800/100 mg twice-a-day, it was shown that a $C_{through}$ >500 ng/ml was associated with increased toxicity, which included nephrotoxicity but also cutaneous toxicity [183]. Burger et al showed that the indinavir concentration at 2 hours post-ingestion ($\approx C_{max}$) should be below 10000 ng/ml in patients receiving indinavir/ritonavir 800/100 mg twice-a-day (n=17) and below 7500 ng/ml in patients receiving receiving indinavir 800 mg three-times-a-day (n=19) to prevent nephrotoxicity [137]. It was not possible to establish a concentration-toxicity association (C_{min} and toxicity) in the MaxCmin trials with patients receiving indinavir/ritonavir 800/100 mg twice-a-day (n=83) [VIII]. Despite very high C_{min} at week 4 (median: 1553 ng/ml), only four patients experienced renal adverse events grade 3/4; two below and two above the median indinavir C_{min} [VIII]. One plausible explanation is that in the MaxCmin1 trial, which was a prospective study, patients received instructions on fluid intake before treatment was initiated and continuously through the study [VIII]. This could influence the incidence of indinavir-associated urological toxicity in comparison with the referenced retrospective studies [24, 183]. Attempts to reduce indinavir-associated toxicity by dosage reductions to 600 or 400 mg of indinavir in combination with ritonavir 100 mg has apparently reduced both toxicity and indinavir concentrations considerably (e.g. study no. 7, 16 and 17, Table 3) [IV, 139, 184-187]. However, most of these studies have methodological problems, e.g. not randomised, lack of control group or retrospective, which makes definitive conclusions inappropriate. One study was an open-label cross-over study (from indinavir 800 mg three-times-a-day to indinavir/ritonavir 400/100 mg twice-a-day) in patients (n=20) with plasma HIV RNA <200 copies/ml [147]. The study was primarily evaluating efficacy and not tolerability but it was reported that no changes in lipids, creatinine, AST or ALT were observed and that only two patients discontinued treatment because of indinavir-associated toxicity.

Nelfinavir

No convincing concentration-toxicity associations have been established with nelfinavir although some studies have tried to link diarrhoea with either very low or very high concentrations [43, 188]. In a study in HIV-infected patients receiving nelfinavir 1250 mg twice-a-day, pharmacokinetic enhancement with ritonavir increased the $C_{through}$ of nelfinavir by 51% but this did not result in significant changes of adverse events (diarrhoea or lipid levels) over 24 weeks [VII]. In another study, gastrointestinal tolerability, including diarrhoea, was improved with a new formulation of the nelfinavir tablet, although there was bioequivalence between the old and the new formulation [189]. One study found that high concentrations of nelfinavir ($C_{through}$ >3300 ng/ml) was an independent risk factor for lipodystrophy [190]. However, patients with high nelfinavir $C_{through}$ were more likely to receive stavudine, which has also been associated with lipodystrophy, compared to patients with lower $C_{through}$, which could be a confounding factor [190, 191]. The observation has not been confirmed by others.

Amprenavir

No associations between the amprenavir C_{min} and toxicity have been described but in a single study with different doses (300-1200 mg twice-a-day) of amprenavir, a C_{max} above the median (not specified) was associated with headache and circumoral paresthesia (n=42) [39].

Lopinavir

Lopinavir concentrations have been associated with changes in lipid levels (total fasting cholesterol and triglyceride) in a study by Gutierrez et al (n=19) [192]. In this study, three patients, all with a lopinavir concentration above 8000 ng/ml (mean 9710 ng/ml), developed a grade 3 lipid elevation, whereas this was not observed in the rest of the patients (mean 6090 ng/ml). However, four of the remaining patients (n=16) had a lopinavir concentration above 8000 ng/ml. Other small studies (n<30) have shown similar trends but it has not been possible to demonstrate this in two larger studies, the RADAR study and the MaxCmin trials (n=55 and n=70) [VIII, 193, 194]. There was a shared opinion in the discussions of these studies that recommendations to reduce the lopinavir dose in case of lipid elevations based on lopinavir concentrations need further investigation. The lopinavir and ritonavir concentration has been shown to be highly correlated and the association between lopinavir concentrations and lipid changes could be confounded by the corresponding

impact of ritonavir [VIII]. Although the concentrations of ritonavir are relatively low when used as a pharmacokinetic enhancer, it has been shown that 300 mg of ritonavir twice-a-day can elevate triglyceride levels significantly within 2 weeks [VIII, 96]. As with nelfinavir, the $C_{through}$ of lopinavir has been correlated with changes in body fat composition, with eight out of nine patients above 8000 ng/ml loosing more than 5% of limb fat after 48 weeks in a prospective study (n=22) [195].

Atazanavir

In the study describing an association between the atazanavir $C_{through}$ and efficacy (discussed earlier), an association with elevations of total bilirubin, which was mainly unconjugated, was also demonstrated. Elevations of total bilirubin were usually only modest causing low-grade adverse events. Total bilirubin >2.5 mg/dl (≈43 µmol/l) was observed in 0%, 17% and 40% of patients with an atazanavir $C_{through}$ of <150 ng/ml, 150-850 ng/ml and >850 ng/ml, respectively [180]. A study by Nóvoa et al confirmed the association and also showed that the MDR1 3435 CC genotype (associated with high P-glycoprotein expression) was associated with high atazanavir concentrations and total bilirubin elevations [196].

To summarise regarding the importance of PI concentrations on efficacy and toxicity: associations have been established with regard to efficacy for all the PIs, both in PI-naïve patients but also in some cases with experienced patients. Unfortunately, methodology is not always comparable and associations are not demonstrated in all studies (discussed below). Toxicity associations have been much more difficult to establish. Suggested minimum effective concentrations (MEC) in PI-naïve and experienced patients and an indinavir toxicity limit, based on references in text, are presented in Appendix 2.

PROTEASE INHIBITORS AND THERAPEUTIC DRUG MONITORING

With therapeutic drug monitoring (TDM), the concentration of a drug is measured and related to a reference value (e.g. MEC), interval or toxicity limit. Subsequently, the result is used to individualise and optimise therapy by dose adjustments or other measures, e.g. pharmacokinetic enhancement, in case of too high or too low concentrations. The rationale behind TDM and the conditions for PI TDM in HIV antiretroviral therapy (Table 5) has been addressed in several reviews [197-199]. One crucial condition, however, which is rarely addressed, is the aspect of the quality of information obtained from the patient and patient adherence (discussed below). Information has to be reliable to apply TDM; if not, decisions based on PI concentration measurements can have adverse effects on both efficacy and toxicity.

Another condition, which is repeatedly addressed, is the association between effect (efficacy and toxicity) and PI plasma concentrations (Table 5). The discrepancy between PI intracellular and plasma concentrations observed in some studies, e.g. with indinavir, has been used as an argument to discard plasma concentrations as a mean to optimise therapy [128]. However, the influence of intracellular protein binding and drug transporters on PI intracellular concentrations is far from fully elucidated, and currently it has not been shown that the PI intracellular concentration is a better marker of effect than the PI plasma concentration. Another, more authoritative argument, which was presented in a leading article about TDM and HAART (Drugs, 2003) by Aarnoutse et al is that "… it should be noted that relationships between plasma PI and NNRTI concentrations and response have all been established by measuring total drug concentrations." and although he was addressing protein binding, it is important to have this in mind when discussing the discrepancy between intracellular concentrations and plasma concentrations [198]. As discussed earlier, a difference in the intracellular/plasma concentration ratio between indinavir co-administered with or without ritonavir has also been demonstrated, which might explain the difference in MEC of the two different regimens [137]. This point underlines the need for research in PI intracellular concentrations but again this is not an appropriate argument to discard plasma concentrations in favour of intracellular concentrations. In the future, with the help of pharmacogenetics, it might be possible to identify patients in which the association between PI plasma concentrations, PI intracellular concentrations and effect (efficacy/toxicity) is not as straightforward as in others, caused by e.g. drug efflux or influx transporters.

Table 5. Conditions for protease inhibitor TDM.

I	Effect (efficacy and toxicity) is associated with PI concentrations
II	The PIs should have a narrow therapeutic index
III	Large inter-individual variability in PI concentrations during steady state circumstances
IV	Low intra-individual variability in PI concentrations during steady state circumstances
V	Lack of good clinical or other laboratory parameters to guide PI therapy
VI	Ability to intervene in case of too high or low PI concentrations
VII	Availability of accurate, precise and specific assays to measure PI concentrations

TDM: therapeutic drug monitoring. PI: protease inhibitor.

There are two other concerns against the use of TDM in PI therapy. One (minor) has been the lack of clear-cut efficacy limits or MECs as discussed earlier with the different approaches that have been used in the studies investigating PI concentration-efficacy associations. E.g. which MEC for amprenavir should be recommended (Figure 3), EC_{50}, EC_{90} or EC_{99}, with a more than 7-fold difference between the EC_{50} and EC_{99} (87 ng/ml and 658 ng/ml)?

The other concern (major) is the lack of well performed, randomised, controlled trials to document the effect of TDM in a clinical setting, i.e. fewer virological failures (and possibly toxicity) in a concentration-controlled arm versus standard of care. Although the ATHENA study (2003) including treatment-naïve HIV-infected patients receiving nelfinavir or indinavir (partly co-administered with ritonavir) was a proof of the TDM concept, it is not reasonable to extrapolate the results from this study to other PIs [200]. Some randomised TDM trials have shown no effect of TDM (Pharmadapt and Genophar) and a number of prospective studies have not been able to demonstrate an association between effect and PI concentrations (MaxCmin trials) [VIII, 201, 202]. The PharmAdapt (n=183) and GENOPHAR (n=134) studies included experienced patients but most of the target MECs were lower than the MECs which are usually applied in naïve patients. Also, the studies were short-term (12 weeks) and both of these two aspects might have underestimated the possible benefits of TDM. As already described, no association between the $C_{through}$ and efficacy could be demonstrated in the MaxCmin trials [VIII]. This could very well be explained by the fact that the $C_{through}$s were very high with 98.2% (277/282) of all the $C_{through}$s above the MEC in PI-naïve patients, and although 49-60% of the patients were protease inhibitor experienced patients, this did not impact overall results. The results are also partly explaining why TDM in PI therapy is not investigated in randomised controlled trials including PI-naïve patients. The PI regimens, which are used currently (2007), with co-administration of ritonavir, have pharmacokinetic capabilities which are less likely to result in concentrations below the MEC (Appendix 1 and 2), which means that studies have to include many patients to show a beneficial effect of TDM.

Whereas MECs determined from wild type virus can be applied in most PI-naïve patients, this is not the case with PI-experienced patients (Appendix 2) where MECs will increase depending on the number and significance of PI-associated resistance mutations (PAMs). It is possible to overcome PI resistance to a certain point, depending on the number of PAMs and the PI, by increasing the dose/concentration of the PI [203]. To increase the likelihood of therapeutic success, the combination of pharmacokinetic and phar-

macodynamic parameters (IQs) in PI-experienced HIV-infected patients has received some attention. However, the majority of presented IQ studies so far have been retrospective and no interventions based on the IQ have been performed. The IQ is the $C_{through}/IC_{50}$ of the HIV phenotype in question, which is why it is also named the PIQ (phenotypic IQ). The GIQ (genotypic IQ) is simply the $C_{through}$/No. PAMs. The PIQ is based on phenotypic resistance testing, which involves complicated *in vitro* systems, needs correction for *in vivo* protein binding, is more expensive, time consuming and less reproducible compared with genotypic resistance testing (sequencing of the HIV protease gene) which is the basis of the GIQ [78]. Genotypic resistance testing, however, is less likely to detect minority HIV populations with different resistance patterns, and consequently the GIQ will perhaps not apply to the entire HIV population. Further, the GIQ does not take into account the differences in impact of each PAM, and hence it is a more rough estimate than the PIQ but also more robust and reproducible. In a review by Hoefnagel et al, the role of the IQ in HIV therapy was discussed by systematically evaluating all studies addressing the use of IQs in HIV-infected patients [78]. Ten studies, including a metaanalysis, investigated the PIQ. Eight studies presented a conclusion regarding the predictive value of the PIQ of which five (63%) were in favour of the PIQ. Fifteen studies (one excluded because of missing data) addressed the use of the GIQ in saquinavir (n=2), amprenavir (n=4), lopinavir (n=5) and atazanavir (n=4) therapy involving 925 patients. Twelve studies (80%) identified the GIQ as predictive of virological response. In the review conclusion it was stated that prospective studies were needed to evaluate if the GIQ could be helpful in overcoming resistance to PIs, e.g. in patients with virological failure or in patients with repeatedly plasma HIV RNA just above 20 copies/ml during PI therapy. In patients harbouring HIV with complete resistance to certain PIs, it is not possible to achieve therapeutic success by increasing the PI dose/concentration. However, it could still be important to address the PI concentration. If the concentration is considerably lower than normal (pharmacokinetic orphans), it could be helpful to focus on the pharmacokinetics of second line antiretroviral drugs to optimize therapy, as e.g. NNRTIs, new PIs (tipranavir), CCR5 antagonists and integrase inhibitors are also metabolised by CYP3A4.

SPECIFIC APPLICATIONS OF PROTEASE INHIBITOR TDM

Whereas the prevalence of patients with low PI plasma concentrations on a population basis will be small (<5-10%), the prevalence will be higher in some populations. Several applications of PI TDM have been suggested in U.S. and European guidelines [204, 205]. TDM is encouraged in HIV-infected patients in case of toxicity; new co-administered drugs (suspected or unknown drug-drug interactions); experimental combinations of antiretroviral drugs including once-a-day PI administration (risk of very low C_{24}s) or PI double boosting; gastrointestinal disease; pregnancy or children (considerable inter- and intra-individual variation). Adherence to PI therapy has, by some, been considered as an application of TDM (or DM in this case). The limitations are obvious because of the short $t_{½}$ of some PIs, e.g. lopinavir/ritonavir (5-6 hours, Appendix 1), which means that it is only the last dosing interval which is evaluated. However, repeated unannounced random PI concentration measurements have been shown to predict virological response and accordingly very low PI drug concentrations could promote consultations with patients to improve adherence [206]. The long-term pharmacokinetics of PI therapy has also been proposed as an application of TDM, following a study by Gisolf et al from 2000, showing decreasing saquinavir concentrations over time (40 weeks) [113]. More recent studies (Table 2) have not been able to confirm or demonstrate any significant decline in PI concentrations, and hence this is probably not a reasonable application for TDM [VII, VIII, 207].

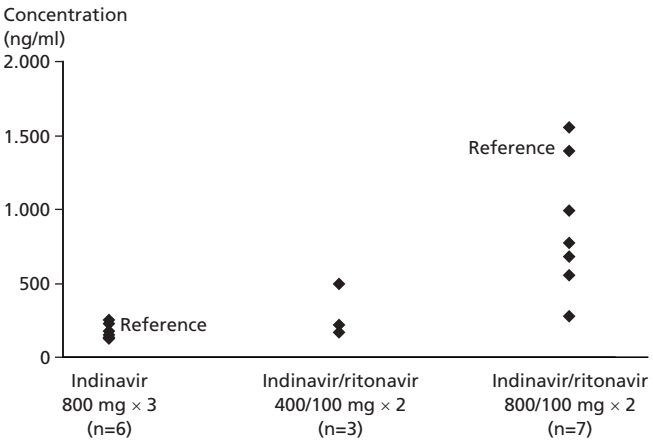

Figure 4. Indinavir $C_{through}$ from the studies in Table 3 and reference indinavir $C_{through}$ from Appendix 1.

GENERAL CONSIDERATIONS OF PROTEASE INHIBITOR TDM

Although a PI concentration-toxicity association has been established or is plausible, it should still be evaluated on an individual basis if dose reductions are necessary as some of the side effects are not serious or transient in nature (e.g. high atazanavir $C_{through}$ and elevated total bilirubin or amprenavir C_{max} and headache/circumoral paresthesia). In contrast, dose reductions would by some be considered an option in case of very high concentrations even though a concentration-toxicity association has not been established. However, this approach should also be evaluated thoroughly on an individual basis, as there are no studies to support this strategy. If dose reductions are performed, studies have demonstrated the possible importance of TDM after dose changes as it is difficult to predict the effect of dose changes, demonstrated in Figure 4, where a 2-fold dose decrease (from 800 mg to 400 mg) could result in a 4- to 6-fold $C_{through}$ decrease. Further, PI concentration decreases should always be evaluated in relation to the MEC to withhold efficacy. In general, dose changes, including dose increases, should be performed with the smallest margin possible to avoid too high or too low concentrations, e.g. as demonstrated in our own study with the disproportionate increase in delavirdine $C_{through}$ of 472% despite a dose increase of only 66% [VI].

In summary, since the introduction of pharmacokinetic enhancement, PI TDM has become less relevant in treatment-naïve patients receiving their first PI regimen as most patients achieve $C_{through}$s several folds higher than the MEC. In PI-experienced patients, the combination of TDM and IQs, especially the GIQ, seems to be a promising tool, but prospective studies are needed. In some patients with certain conditions or in certain circumstances (toxicity, drug-drug interactions, gastrointestinal disease, pregnancy or children) TDM might be of benefit, although no studies, apart from toxicity, have investigated these patients specifically in randomised TDM trials.

GENERAL CONCLUSIONS AND PERSPECTIVES

The presented studies and review demonstrate: 1) that it is feasible to measure PI plasma concentrations in a clinical setting with precision and accuracy, and that PI concentrations are very stable during different circumstances *ex vivo* [II]; 2) that PI plasma concentrations display limited long-term intra-individual variation but considerable inter-individual variation [III-VIII]; 3) that PI plasma concentrations display considerable intra-individual variations between morning and evening and in the case of drug-drug interactions [I, III, V]; 4) that PI drug-drug interactions can be unpredictable and adverse but also that PI drug-drug interactions can be exploited to increase PI concentrations or decrease PI dose [III-VIII]; 5) that increases in PI plasma concentrations can enhance efficacy but also that decreases can reduce toxicity [IV, VIII]; 6) that the concentration-efficacy associations which have been established by

others can be confirmed in clinical trials but that concentration-toxicity associations are more difficult to establish and confirm [VIII].

Some of these aspects of PI plasma concentration variation could have potential consequences for HIV antiretroviral therapy, i.e. efficacy and possibly toxicity. The application of TDM can (probably) be used to increase the likelihood of therapeutic success, i.e. secure or increase efficacy and avoid or decrease toxicity, but it cannot guarantee it.

To reach credible conclusions concerning the possible advantages of PI TDM versus standard of care in special populations or situations, it would be necessary to establish an international multicenter collaboration and trial to assemble enough patients. However, it seems that the time for such a study has already passed as TDM has become an integrated part of HIV antiretroviral therapy in several European countries (United Kingdom, The Netherlands, Italy, France and Sweden) despite the lack of evidence from randomised, controlled trials [205, 208-211].

The use of TDM and IQs in PI-experienced patients is from a theoretical point of view the ultimate combination of pharmacokinetic and pharmacodynamic data, but as often requested, prospective randomised controlled trials are needed to support this strategy. Some studies are currently (2007) in progress, e.g. the ACTG study 5146.

What about PIs in the future? The PIs, especially atazanavir and lopinavir co-administered or formulated with ritonavir, in combination with NRTIs are still considered first line therapy in PI-naïve but also treatment-naïve patients, although efavirenz is currently (2007) the drug of choice supported by data from clinical trials. A new PI, tipranavir, has been approved for the treatment of HIV-infected patients harbouring virus with resistance towards multiple PIs. Tipranavir is a strong inducer of CYP3A4 and has to be co-administered with ritonavir 200 mg twice-a-day to compensate for the inducing effect but the potential for adverse drug-drug interactions still exists. Concentrations of the PIs, e.g. saquinavir, amprenavir and lopinavir, have been shown to decrease considerably when co-administered with tipranavir [212]. Two other twice-a-day PIs, darunavir (TMC114) and brecanavir (GW640385), are also metabolised by CYP3A4 and co-administered with ritonavir [213, 214]. The accelerated drug approval process of antiretroviral drugs, including PIs, has the consequence that long-term experience; knowledge of drug-drug interactions or pharmacokinetics in special populations is limited. TDM has been suggested as part of the post marketing surveillance for other drugs to identify pharmacokinetic problems as early as possible [215]. PI drug-drug interactions with the NNRTIs efavirenz and nevirapine are well known, but new NNRTIs, the CCR5 antagonists (e.g. maraviroc, metabolised by CYP3A and a P-glycoprotein substrate) and the integrase inhibitors (e.g. GS-9137/elvitegravir, metabolised by and a possible inducer of CYP3A) are in development and available through expanded access programs [216, 217]. Pharmacokinetic data regarding drug-drug interactions between these new drugs and the PIs are naturally sparse but could be supplemented by TDM.

Although many promising antiretroviral drugs are in development, the primary goal in the treatment or eradication of HIV is still a vaccine but progress to produce an effective vaccine is slow. In the meantime, known therapeutic regimens must continuously be optimised to improve survival and quality of life.

SUMMARY

Since the introduction of the HIV protease inhibitors in 1995, considerable progress has been made in the treatment of HIV-infected patients. However, treatment has not been without problems. Studies have demonstrated associations between protease inhibitor concentrations and efficacy and in some cases toxicity. As considerable inter-individual and intra-individual variations of protease inhibitor concentrations have been observed, it has been questioned to what extent this has clinical implications with regard to efficacy and toxicity? As a consequence the use of protease inhibitor concentration measurements to optimise HIV antiretroviral therapy (therapeutic drug monitoring – TDM) has been suggested. The objectives of this study, initiated in 2000, were: to establish a method for the simultaneous measurement of the available protease inhibitors; to explore the pharmacokinetics of the protease inhibitors in clinically relevant situations and in this context; to consider the applicability of TDM in protease inhibitor therapy.

The presented studies and review demonstrate: 1) that it is feasible to measure protease inhibitor plasma concentrations in a clinical setting with precision and accuracy, and that protease inhibitor concentrations are very stable during different circumstances *ex vivo*; 2) that protease inhibitor plasma concentrations display limited long-term intra-individual variation but considerable inter-individual variation; 3) that protease inhibitor plasma concentrations display considerable intra-individual variations between morning and evening and in the case of drug-drug interactions; 4) that protease inhibitor drug-drug interactions can be unpredictable and adverse but also that protease inhibitor drug-drug interactions can be exploited to increase protease inhibitor concentrations or decrease protease inhibitor dose; 5) that increases in protease inhibitor plasma concentrations can enhance efficacy but also that decreases can reduce toxicity; 6) that the concentration-efficacy associations which have been established by others can be confirmed in clinical trials but that concentration-toxicity associations are more difficult to establish and confirm.

The experiences with protease inhibitor therapy and the understanding of protease inhibitor pharmacokinetics have resulted in new treatment principles and the development of new and better protease inhibitors. Most patients achieve concentrations several folds higher than the minimum effective concentration with the regimens that are used currently (2007). As a result TDM in protease inhibitor therapy has become less relevant in HIV-infected patients receiving their first protease inhibitor. In protease inhibitor experienced patients, harbouring HIV with varying degrees of resistance/reduced susceptibility to protease inhibitors, the combination of TDM and genotypic resistance testing, seems to be a promising tool, but prospective studies are needed. In some patients with certain conditions or in certain circumstances known to be associated with considerable inter-individual or intra-individual variation of protease inhibitor concentrations (drug-drug interactions, gastrointestinal disease, pregnancy or children) TDM might also be of benefit. However, no studies have investigated these patients specifically in randomised TDM trials.

ABBREVIATIONS

C_{min}	Minimum plasma drug concentration*
C_t	Plasma drug concentration at a specified time t after the administration of a given dose*
$C_{through}$	Plasma drug concentration at the end of a dosing interval directly before the next*

*) The three abbreviations are often used synonymously although the definitions are different.

ACTG	AIDS Clinical Trials Group
AIDS	Acquired immunodeficiency syndrome
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
AUC	Area under the curve
CCR	Chemokine coreceptor
CD	Cluster of differentiation
CV	Coefficient of variation
CYP	Cytochrome P450
DNA	Deoxyribonucleic acid
EC	Effective concentration

E_{max}	Maximum effect
EMEA	European Agency for the Evaluation of Medicinal Products
FDA	U.S. Food and Drug Administration
GIQ	Genotypic inhibitory quotient
HAART	Highly active antiretroviral therapy
HGC	Hard-gel capsule
HIV	Human immunodeficiency virus
HPLC	High-performance liquid chromatography
IC	Inhibitory concentration
IQ	Inhibitory quotient
MDR	Multidrug resistance
MEC	Minimum effective concentration
MRP	Multidrug resistance associated protein
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NRTI	Nucleoside reverse transcriptase inhibitor
NtRTI	Nucleotide reverse transcriptase inhibitor
PAM	Protease inhibitor associated resistance mutation
PBMC	Peripheral blood mononuclear cell
PI	Protease inhibitor
PIQ	Phenotypic inhibitory quotient
RNA	Ribonucleic acid
ROC	Receiver operating characteristic
SGC	Soft-gel capsule
TDM	Therapeutic drug monitoring
$t_{1/2}$	Elimination half-life
UV	Ultraviolet

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APPENDICES
PROTEASE INHIBITOR PHARMACOKINETIC PARAMETERS

Appendix 1. Protease inhibitor pharmacokinetic parameters. Adopted from the product information, European Medicines Agency (EMEA) [37].

Protease inhibitor	Dose	Bioavailability (%)	C_{max} (ng/ml)	C_{min} (ng/ml)	t_{max} (h)	$t_{½}$ (h)	Plasma protein binding (%)	Metabolism: cytochrome P450 isozyme
Saquinavir HGC	600 mg × 3	4	197	75	2.4-3.8	NR	97	CYP3A4
Saquinavir SGC	1200 mg × 3	NR	2181	216	NR	NR	97	CYP3A4
Saquinavir HGC[a]	1000 mg × 2	NR	2623	371	NR	NR	NR	-
Ritonavir	600 mg × 2	NR	11200	3700	4	3-5	98-99	CYP3A4 and 2D6
Indinavir	800 mg × 3	65	9514	227	0.8	1.8	61	CYP3A4
Indinavir[a]	800 mg × 2	NR	11657	1395	NR	NR	NR	-
Nelfinavir	1250 mg × 2	NR	4000	700-2200[b]	2-4	3.5-5	≥98	CYP3A4 and 2C19
Amprenavir	1200 mg × 2	NR	5360	280	1-2	7.1-10.6	90	CYP3A4
Fosamprenavir[a]	700 mg × 2	NR	6080	2120	1.5	15-23	90	CYP3A4
Lopinavir[a]	400 mg × 2	NR	9600	5500	4	5-6	98-99	CYP3A
Atazanavir[a]	300 mg × 1	NR	5233	862	3	8.6	86	CYP3A4

HGC: hard-gel capsule. SGC: soft-gel capsule. NR: not reported.
a) In combination with ritonavir 100 mg × 1-2.
b) Evening and morning $C_{through}$. Nelfinavir displays significant diurnal variation [26].

PROTEASE INHIBITOR MINIMUM EFFECTIVE CONCENTRATIONS

Appendix 2. Protease inhibitor minimum effective concentrations in naïve and experienced patients and an indinavir toxicity limit. Suggestions based on references in text.

Protease inhibitor	Naïve patients[a] (ng/ml)	Experienced patients (ng/ml)	References
Saquinavir	>100	-	[20, 171, 172]
Ritonavir	>2100[b]	-	[21]
Indinavir	>250 (C_{max} <10.000)	-	[137]
Nelfinavir	>1400 (morning) >800 (evening)[c]	-	[26, 176, 177]
Amprenavir	>250	>1250	[39, 178]
Lopinavir	>1000	>5700	[161, 179]
Atazanavir	>150	-	[180]

a) Concentrations have been determined from wild type virus without protease inhibitor-associated resistance mutations or reduced susceptibility.
b) Administered as the only protease inhibitor.
c) Nelfinavir displays significant diurnal variation [26, 176].